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THE UNIVERSITY OF ALBERTA

THE ROLE OF PHOTOPERIOD AND TWILIGHT IN THE CONTROL OF LOCOMOTORY RHYTHMS IN THE LAKE CHUB, *COUESIUS PLUMBEUS* (AGASSIZ)

by

© MARTIN IMANTS KAVALIERS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1978

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The role of photoperiod and twilight in the control of locomotory rhythms in the lake chub, *Couesius plumbeus* (Agassiz)," submitted by Martin Imants Kavaliers in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Date ....

ABSTRACT

The roles of twilight portions of photoperiods, dawn and dusk, in the entrainment of the locomotory activity of the cyprinid *Couesius plumbeus* (lake chub) were examined.

When *C. plumbeus* were kept under photoperiods with duplicated natural twilights, the phasing of the beginning of their activity, relative to sunrise, displayed a bimodal annual pattern that was correlated with seasonal changes in twilight duration and spectral characteristics. The end of maximal activity, relative to sunset, had a unimodal pattern that was consistent with seasonal changes in daylength and total light energy in twilight. No annual patterns in the phasing of activity were evident in fish entrained by seasonally appropriate photoperiods that excluded twilights.

The locomotory activity of *C. plumbeus* consisted of a number of diverse behavioural components. The locomotory behaviours were not equivalent to the circadian activity parameters of birds and mammals normally investigated in the laboratory. The overall activity of *C. plumbeus* was distributed in sub-components or 'bouts' of 6 minutes to 3.5 hours total duration. These bouts fit a non-random Gamma (γ) frequency-duration distribution. Under simulated and natural photoperiods there were significant seasonal changes in mean activity bout length, 1.4 and 1.7 hrs for winter and summer, respectively, as well as distribution characteristics. There were no significant seasonal changes under photoperiods without twilights.

A free-running circadian rhythm of locomotory activity of *C. plumbeus* was evident under constant conditions. Fish that were entrained under photoperiods with twilights had significant seasonal changes in period length (tau), 28.0 and 24.5 hours for winter and summer, respectively. Fish that were entrained under photoperiods without twilights did not display any significant seasonal differences in the length of the free-running period. Changes in tau were interpreted as being after-effects of the more stringent and precise entrainment of activity under photoperiods with twilights.

The roles of the various spectral components of twilight in the entrainment of the activity of *C. plumbeus* were examined. Determinations of action spectra for entrainment and behavioural induction were carried out. Rates of energy change of specific spectral regions of twilight, as well as spectral intensity ratios, were used as cues to phase the beginning of activity. Concurrent ablation studies indicated that entrainment was mediated by the lateral eyes, the pineal, and other extra-ocular photoreceptors.

Seasonal and daily changes in twilight components of natural photoperiods functioned as exact cues to phase and entrain the activity of *C. plumbeus*. These entrained behavioural components served as an 'activity template' that could be modified by other stochastic variations in the aquatic environment.

ACKNOWLEDGEMENTS

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I was helped greatly by Mr. W. Roberts who spent many hours in assisting in the collection of fish, and by Mr. E. Whiting who provided limnological data and records from the collecting area.

The parasitology students deserve my gratitude for their friendship, useful comments, good humour, and reminders that there are alternative ways of viewing biological systems.

Thanks are due to Mrs. K. Baert for her skill and useful advice in the preparation of the typescript.

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Chapter I

INTRODUCTION

All organisms show cyclic or rhythmic variations in their behavioural and physiological functions. It is now generally accepted that daily rhythms have an endogenous component that acts as some form of oscillatory system (Bünning 1973; however, see Brown 1976 for an alternative explanation). The most substantial evidence for such an endogenous system is the demonstration of a free-running rhythm under constant conditions which has a period close to, but not exactly, 24 hours. The approximate 24 hour rhythm is termed circadian and the free-running period (FRP) is designated by tau (T).

Circadian processes are synchronized with each other and with the environment on both daily and seasonal bases. Periodic environmental changes that are able to synchronize or entrain different biological cycles are known as Zeitgebers, cues, or entraining agents. For circadian rhythms the major entraining agent is the daily cycle of illumination or photoperiod. Circadian entrainment involves both phase and frequency control. The circadian period is transformed to the period length of the exogenous cycle, T, in a definite phase relationship (Ψ). Photoperiod is the only environmental periodicity that is known to entrain all circadian rhythms. Rhythms of poikilotherms can also be cued by other environmental factors, the most prominent of these being temperature and acoustic cycles (Bünning 1973).

The daily cycle of illumination or photoperiod is composed of daylight and twilight components along with night periods. The majority of laboratory and field studies of photoperiodically entrained activity, which have been primarily carried out with birds and mammals (see review by Daan and Aschoff 1975) have either ignored twilights or only given them cursory attention. Yet there is evidence that twilight is of crucial importance in controlling circadian events. Kavanau and co-workers (Kavanau 1962, 1967, 1968, 1969, 1971; Kavanau and Peters 1974, 1976a,b; Kavanau and Ramos 1975) observed that many mammals are either most active at particular twilight intensities, or use dawn and dusk as cues for setting daily activity. Similar evidence has been obtained for a role of twilight in the activity patterns and community changeovers in coral reef fishes (Hobson 1973).

More theoretical analyses of the roles of twilight in the timing of the activity of birds and mammals were carried out by Wever (1967), Aschoff (1969), and Daan and Aschoff (1975). Their studies showed that twilights were involved in the entrainment process.

Results and conclusions from all these studies are limited by incomplete characterizations of twilight. Seasonal changes in twilight properties other than duration were not considered. Laboratory duplications often were only approximate and poorly defined. Analyses of the roles of twilight in photoperiodic entrainment have been completely neglected with fish.

The present study was undertaken 1) to determine the general roles of twilight in the entrainment of the locomotory activity of the lake chub, *Couesius plumbeus*, and 2) to examine the role(s) of different twilight components in determining the daily periodicity and entrainment of the activity of *C. plumbeus*. Undertaking these objectives also entailed: 1) the examination in detail of the daily periodicity and entrainment of the locomotor activity of *Couesius plumbeus*, and 2) the observation of seasonal changes in the entrainment and activity of the lake chub.

Chapter II

MATERIALS AND METHODS

A. Experimental Animals and Study Area

Couesius plumbeus (Agassiz), the species of cyprinid used in this study, has been described by Scott and Crossman (1973). Its range extends from the northern United States through most of Canada. Typical habitats where it is found are streams, lakes and rivers. Various aspects of the general and reproductive biology are described by Ashan (1966a,b), Brown et al. (1970), Scott and Crossman (1973).

The experimental fish were obtained by bi-weekly seining from the North Saskatchewan river and the confluence of Whitemud Creek at Edmonton, Alberta (53°34'N; 173°29'W) as well as at a site 0.25 km further downstream. These sites are described in Appendix I. In winter all collecting was limited to the latter downstream site as it was the only portion of the river that was not frozen over. A 15 M bag seine (5.0 M bag and 1.0 cm mesh) was used for collecting the fish. Immediately after being caught fish were transported to the University of Alberta and transferred to holding tanks set at predetermined light-dark cycles and water temperatures. The temperature values were based on seasonally appropriate field values listed in Appendix I. The fish were fed daily at a random time with a commercially prepared diet (Purina Trout Chow, Ralston Purina, St. Louis, Missouri). The entire collecting and transport operation usually took less than one hour. Collecting was done at various times of the day and evening to reduce sampling biases for either diurnally or nocturnally active individuals and species of fish. The number(s) of lake chub relative to the total number of fishes and species caught fluctuated monthly. The exact proportions and numbers of fish caught are provided in Appendix I.

C. plumbeus were placed in transluscent, non-reflective opaque, plexiglass tanks (60 x 90 x 30 cm) under a twilight simulator described on page 31. The tanks were fitted with an adjustable water flow (0-50 ml min^{-1}) that could be set to any desired temperature (Appendix I). Normally water temperature was maintained close to that found in the river (± 2°C). Experiments were done with and without gravel substrates present in the tanks. Feeding in the experimental situations was carried out at random times to prevent inadvertent cueing of activity. Fish that were not immediately used for activity determinations were placed in either indoor holding tanks (90 x 90 x 60 cm) set to natural photoperiod lengths (LD, on-off photoperiods) or were held outside in behavioural observation tanks (2.0 m diam; 20 cm depth).

B. Characteristics of Photoperiods and Twilights

Twilight, either at dawn or dusk, is essentially the interval separating daytime illumination from that of night. Photoperiod is a general term that describes the light fraction of the day; it may or may not include twilights. Sunlight, daylight, or daylength is specifically measured from sunrise to sunset.

Irradiance is the measure of light energy that is used to describe photoperiods and twilights in this study. Irradiance is defined as the intensity of a light beam falling on a surface and is expressed in terms of energy per unit area per incident wavelength ($\mu w\ cm^{-2}\ nm^{-1}$). Irradiance was measured at 25 nm intervals (350 to 750 nm) with an ISCO spectroradiometer (Instrument Specialties Company, Lincoln, Nebraska) and a research spectroradiometer (International Optics, California). All energy values presented were measured by the author, unless specifically stated otherwise.

Spectral irradiance, when measured with the detector surface normal to the zenith, provides a reliable estimate of the actual spectral/energy distributions of available light during twilight (McFarland and Munz 1975). For daylight comparable estimates are obtained only when the sun's altitude is fairly high. Therefore, only noon time (1200 hrs Mountain Standard Time (MST)) spectral energy values are presented. Radiance only describes the light energy from a particular portion of the sky. Both energy measures can be presented as integrated energy fluxes over a defined spectral range, e.g. 350-750 nm for 'visible' light. Integrated total 'visible' irradiance is the value used in the plots of total energy that are presented in this study.

Spectral energy can be described in terms of spectral composition (wavelength λ or frequency $V = c^{-1}$) and quantal number (photons $n = E \lambda h^{-1} c^{-1}$) where h = Planck's constant $6,624 \times 10^{-27}$ erg sec^{-1}; c = speed of light (2.96×10^{-10} cm sec^{-1}). Photon number possesses a direct linear relationship to frequency, but is inversely and non-proportional [illegible] d to wavelength. Spectral maxima are shifted to lower freque [illegible] expressed in quantal rather than energy units. Dartnall (1975 [illegible] hat spectral radiation should be preferentially described in photon and frequency units. He suggests that these provide a more reliable indication of the effective or functional characteristics of solar radiation. However, the relative merits of these units still remains moot. In this study light characteristics are primarily described in terms of their wavelengths and spectral energies, these being the calibration units of the spectroradiometer. Occasional references and conversions to photon values are made.

Underwater spectral energy readings were obtained by using the ISCO spectroradiometer with a light probe and cosine collector to collect diffusely distributed radiation. Continuous records of total surface radiation integrated from 200 nm to 0.3 m were also obtained with an Eppley pyranometer. These records were used as a check on the sensitivity of the other detectors and were not used for any analyses.

Representative examples of the 'visible' spectral energy distributions of daylight on clear and overcast days are provided in Figure 1. Overcast days are defined as having complete, unbroken, cloud cover. The spectral energy distribution has a maximum at approximately 550 nm. Cloud cover causes a relative enhancement in the red (600 nm) portion of the spectrum and a relative reduction in the blue (400-500 nm). However, the relative maximum at 550 nm is not completely eliminated. The midday spectral composition remains relatively constant throughout the year. The total 'visible' incident energy and photon numbers, which are functions of the altitude of the sun, display significant seasonal fluctuations (Fig. 2).

The spectral energy distribution present in the night sky is primarily dependent on moonlight (Rozenberg 1966). At Edmonton, total

Fig. 1. Examples of spectral energy distributions (μw cm^{-2} nm^{-1}) at noon (1200 hrs MST). Clear days (———); overcast days (-----), April 23 and April 27, 1975, respectively. Since similar spectral distributions are present throughout the year, only one is presented as an illustrative example.

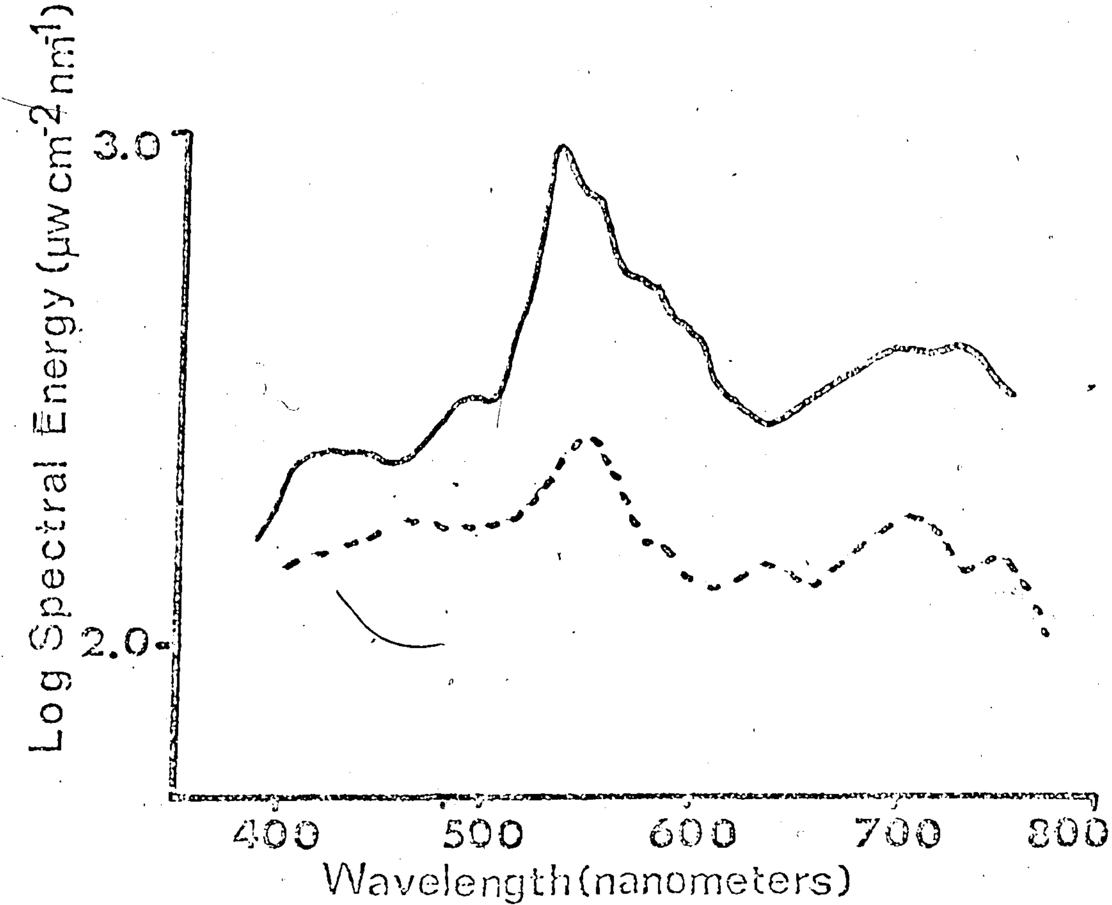
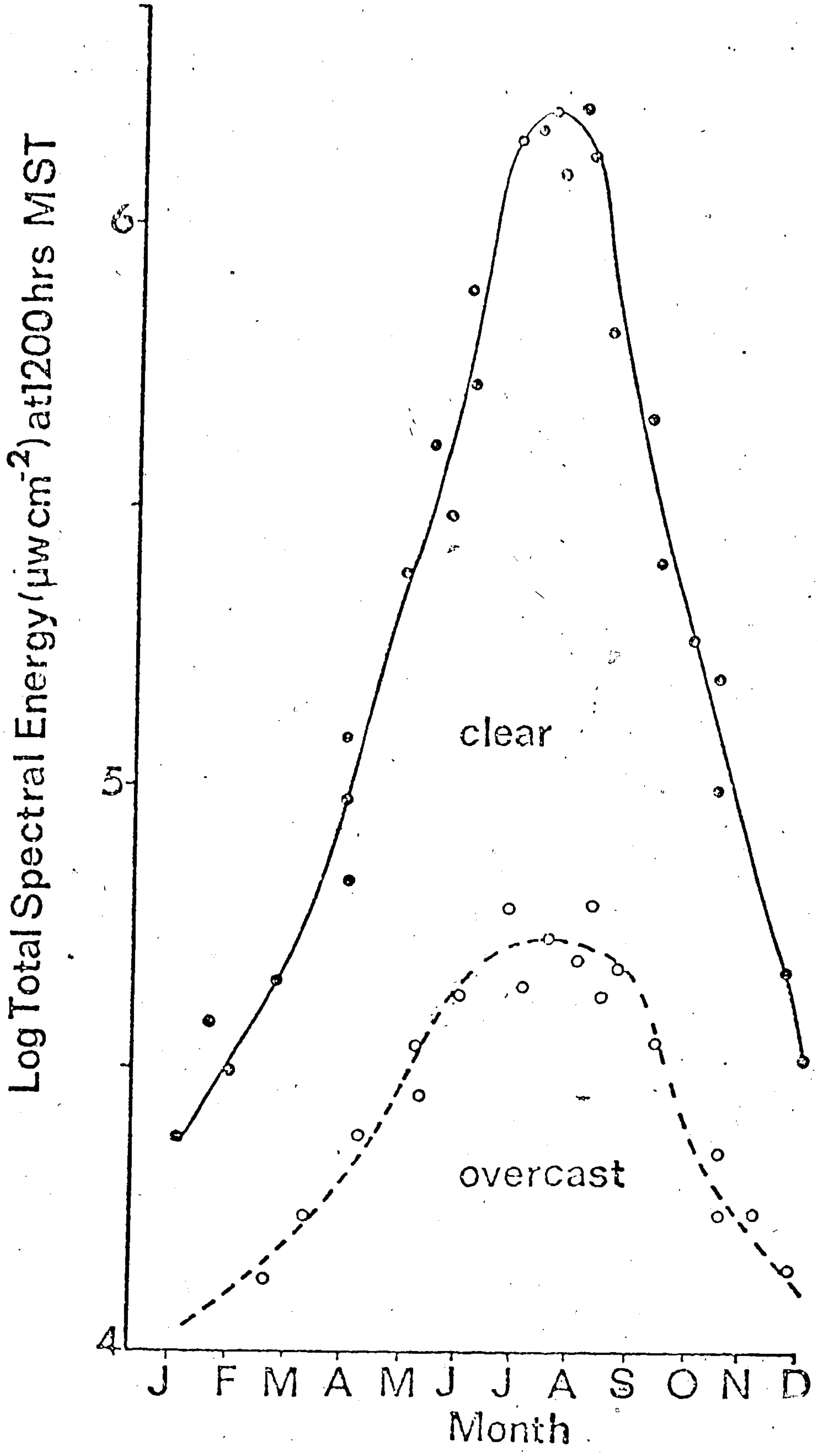

Log Spectral Energy (µw cm⁻² nm⁻¹)
3.0
2.0
400
500
600
700
800
Wavelength (nanometers)

Fig. 2. Annual changes in total spectral energy (350-750 nm; in μw cm^{-2}) at noon (1200 hrs MST). Each point represents the mean of 3-7 days. Data are provided for clear (———) and overcast (-----) days.


Log Total Spectral Energy (μw cm-2) at 1200 hrs MST
6
5
4
clear
overcast
J F M A M J J A S O N D
Month

incident energy varies from approximately 1 x 10^{-4} to 1 x 10^{-2} μw cm^{-2} for moonless and full moon conditions, respectively. In the early part of the night (3-4 hrs after sunset) spectral energy values are maximal at 500-600 nm. This maximum gradually shifts to longer wavelengths, with red (600 nm+) from starlight becoming more predominant in the later portions of the night.

Twilight is produced by the movement of the sun below the horizon with an accompanying scattering sunlight in the earth's atmosphere and attenuation of direct solar radiation. Twilight is arbitrarily divided into civil, nautical, and astronomical portions (Fig. 3) (Rozenberg 1966). These are defined in terms of the position of the sun below the horizon: -6°, -12°, and -18°, respectively, for the aforementioned divisions. At high latitudes, including Edmonton, during the summer (Jun-Aug) there is a continuum of astronomical to nautical twilights without any true night periods. Civil twilight has a bimodal annual pattern, being shortest during spring and fall (Fig. 4).

During dawn there is an alteration of spectral composition from night to that of full daylight, with the reverse processes occurring at dusk. These two processes are basically symmetrical and describing one is sufficient to characterize the other (Rozenberg 1966).

In dawn, during the astronomical to nautical twilight transition, there is initially a rise in energy in the 750 nm region followed by a relative decline in the 400-450 nm region (0.5 x 10^{-4} to 3.5 x 10^{-4}; and 3.6 x 10^{-4} to 8 x 10^{-3} μw cm^{-2} respectively).

At civil twilight light at the 500-600 nm region shows a relative increase and light at 750 nm a relative decline in energy

Fig. 3. 'Schematic' illustration of the arbitrary divisions of twilight after Rozenberg (1966). Sample light energy values ($\mu w\ cm^{-2}\ 550\ nm^{-1}$) from clear mornings and evenings on April 23 and April 24, 1975, respectively, are plotted as a function of the altitude of the sun. The solid line is an eye fit of the individual energy values. Dashed lines indicate time of onset of astronomical twilight (sun -18° from horizon), nautical (-12°), and civil twilight (-6°), and sunrise/sunset (0°).

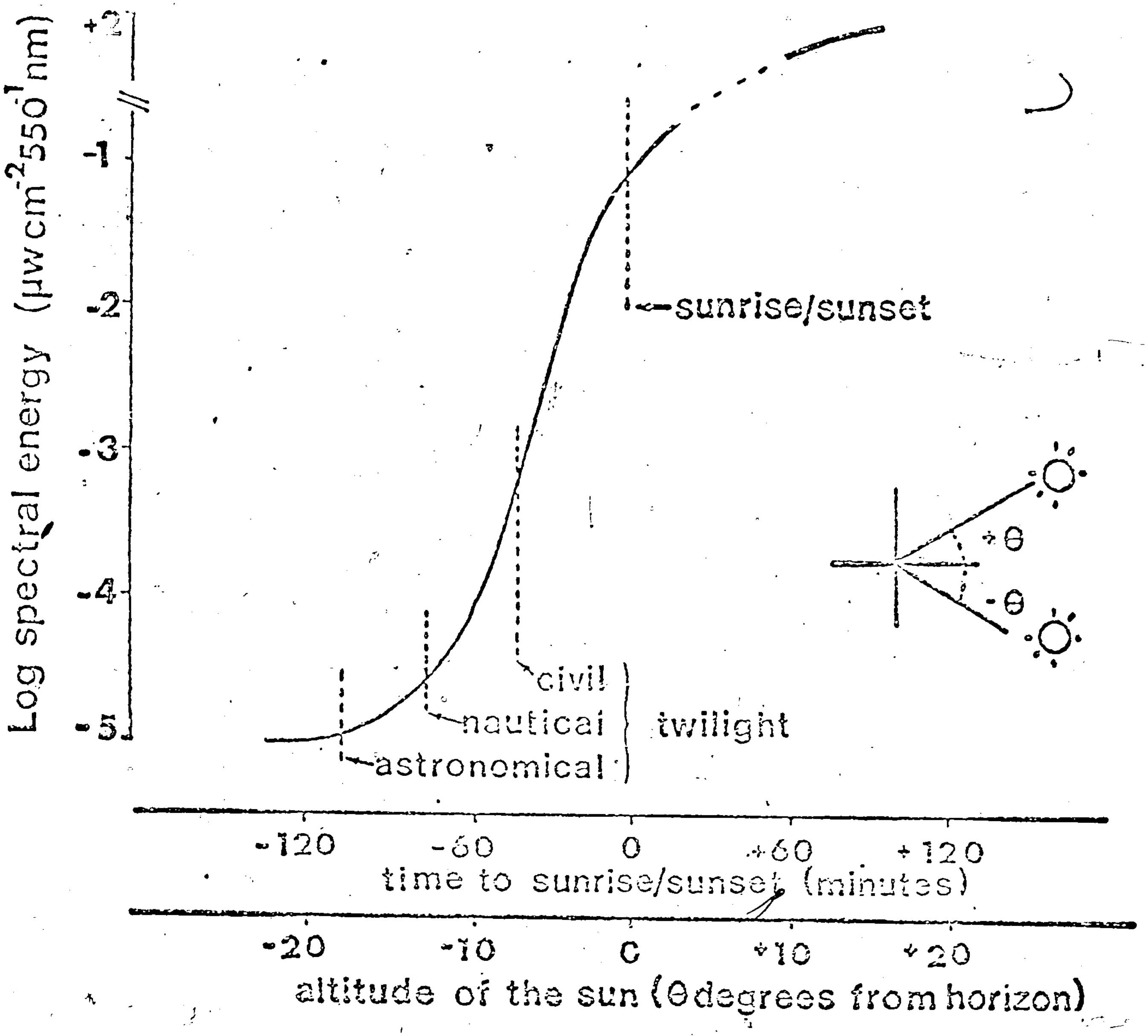
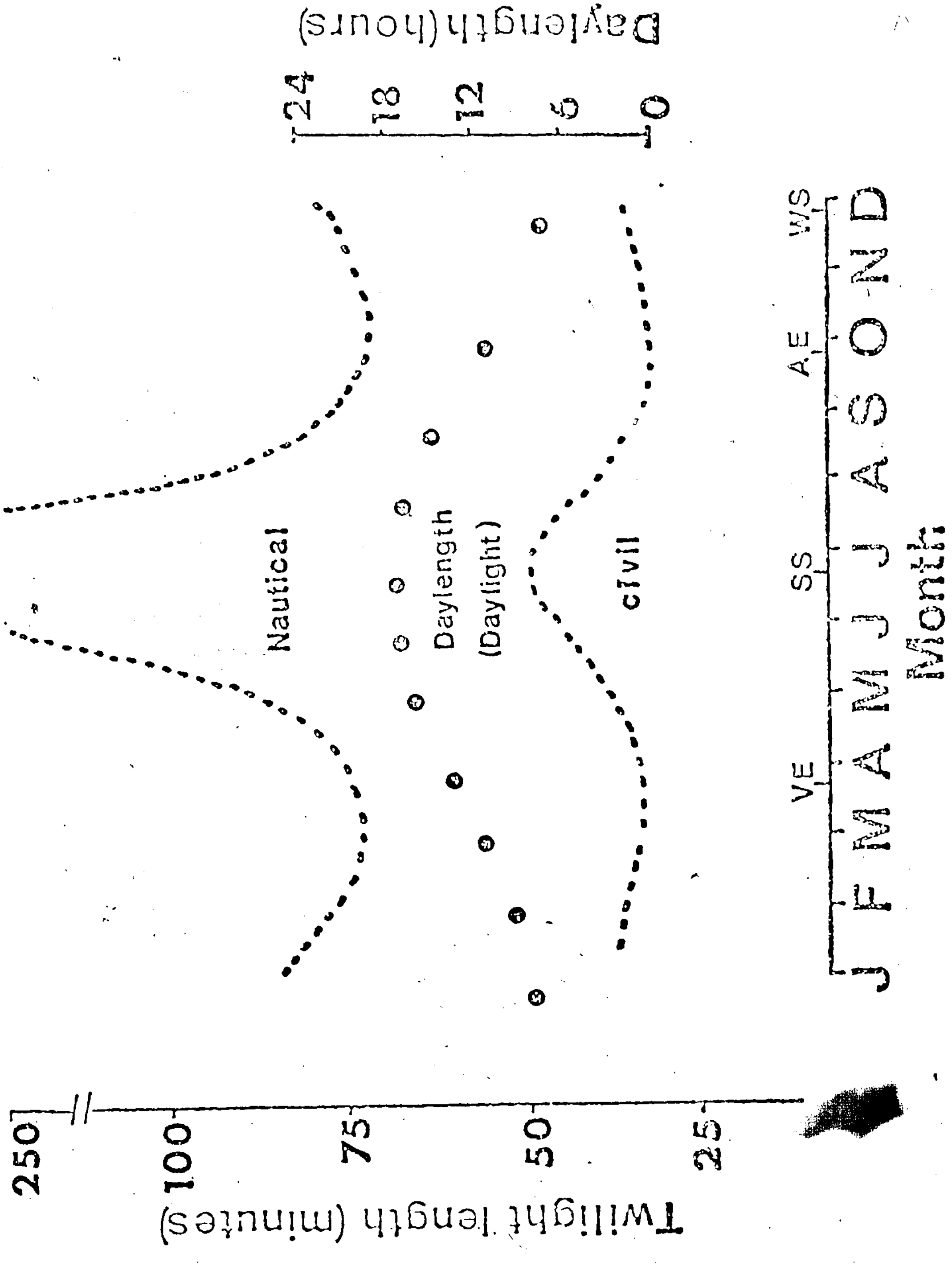

Log spectral energy (μw cm^{-2} 550^{-1} nm)
+2
-1
-2
-3
-4
-5
sunrise/sunset
civil
nautical
astronomical
twilight
+θ
-θ
-120
-60
0
+60
+120
time to sunrise/sunset (minutes)
-20
-10
0
+10
+20
altitude of the sun (θ degrees from horizon)

Fig. 4. Seasonal changes in daylength or daylight (...) and the lengths of civil and nautical twilights (---) at 53° 34' N latitude. Note the bimodal annual patterns in both twilights. WS = winter solstice; VE = vernal equinox; SS = summer solstice; AE = autumnal equinox.


Twilight length (minutes)
250
100
75
50
25
Nautical
Daylength
(Daylight)
civil
Daylength (hours)
24
18
12
6
0
VE
SS
AE
WS
J F M A M J J A S O N D
Month

(Fig. 5). However, the latter is still a prominent component of the spectral composition. With the coming of sunrise the spectrum blends into a full daylight spectrum. The reverse spectral procedures occur at dusk. In all cases there is a reduction in the yellow-orange (550-650 nm) components of the spectrum and relative enhancement of the red and blue (400-500 nm) and to a lesser degree an enhancement of the blue-green portions of the spectrum. The decline in yellow-orange spectral components results from a specific absorption by ozone (Chappuis effect) present in the increased amount of atmosphere through which sunlight must pass before it strikes the surface of the earth (Rozenberg 1966). This results in the relatively increased horizontal 'redness' and 'blueness' from diffuse skylight.

These same general patterns of change occur throughout the year. The exact positions of twilight components relative to sunrise and sunset vary in a bimodal annual pattern that is consistent with the annual duration of twilight and its rate of change. Spring and fall twilights are antipodal in direction but essentially similar in their shift in spectral energy distribution and change. The two equinox twilights, the shortest civil twilights of the year, can be considered as being equivalent in structure and form.

There are daily and seasonal variations in the relative durations and rates of change of the various spectral components of twilight. Spectral, energy, and quantal characteristics do not change at uniform rates during twilight. For example, Figure 6 shows the lower the energy at 510 nm the greater the rate of change in energy. These relationships are only valid for the energy levels found in twilights.

Fig. 5. Spectral composition and energy values ($\mu w\ cm^{-2}\ nm^{-1}$) before and after sunset during civil twilight on a clear day. Values are for June 18, 1974. Similar changes (not illustrated) are also seen at dawn.

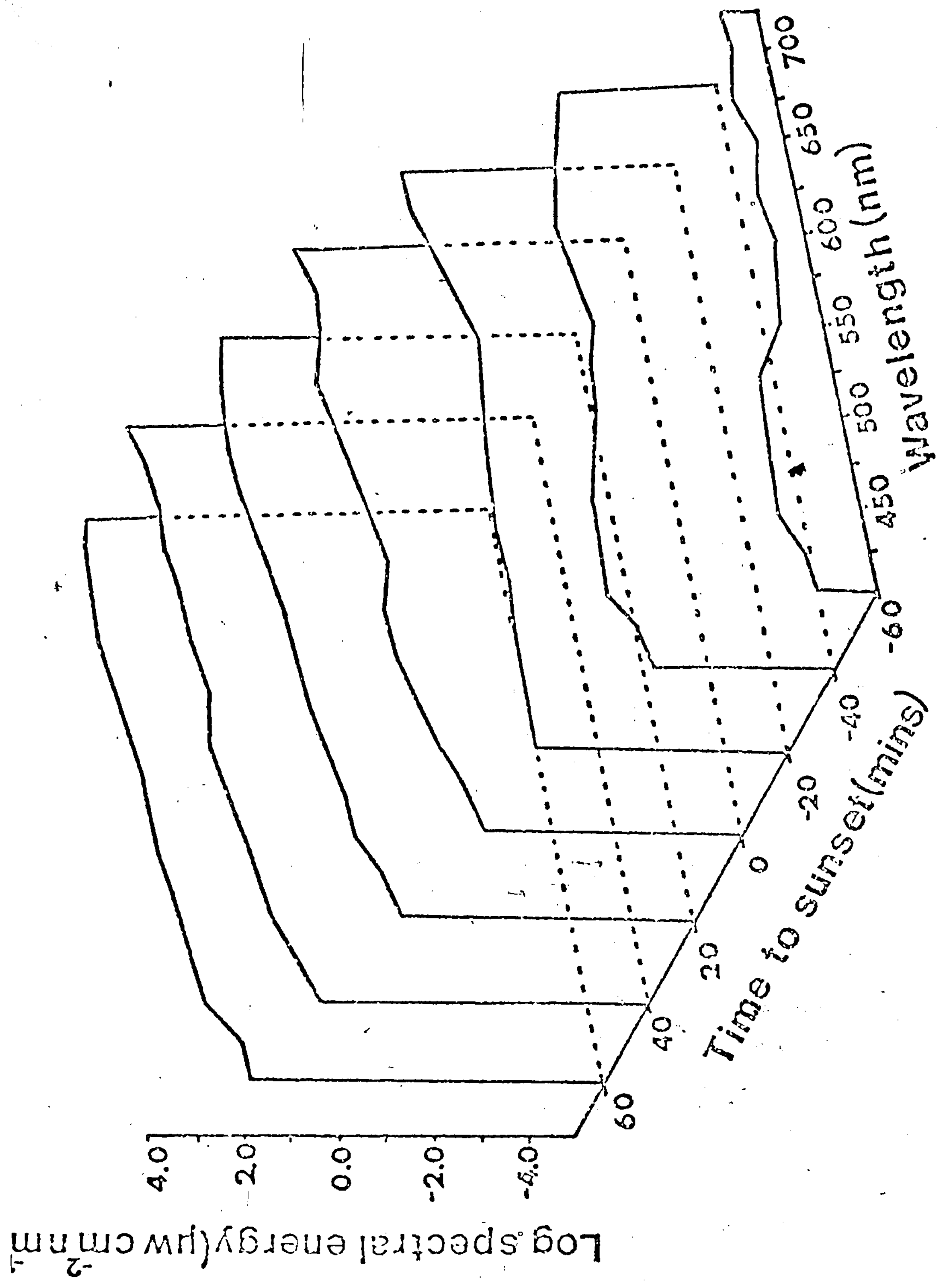
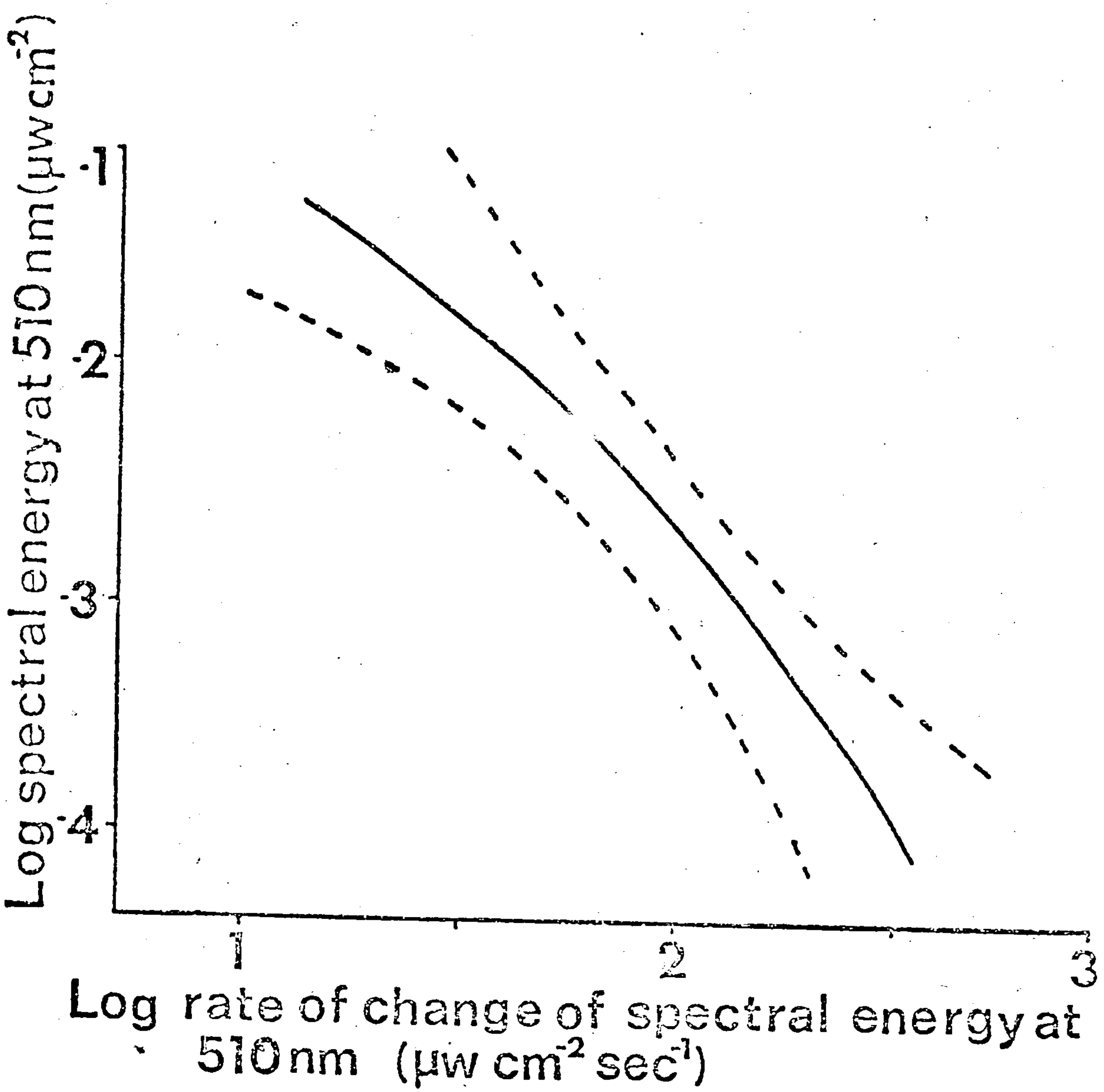

Log spectral energy (μw cm^{-2} nm^{-1})
4.0
2.0
0.0
-2.0
-4.0
60
40
20
0
-20
-40
-60
Time to sunset (mins)
450
500
550
600
650
700
Wavelength (nm)

Fig. 6. Absolute rate of intensity change at 510 nm ($\mu w\ cm^{-2}\ sec^{-1}$) plotted as a function of spectral energy values found during dawn and dusk. The solid line (——) is based on mean rates of change obtained during the course of the year (n = 42). The dashed lines (---) represent the 95% confidence limits of the best fit.


Log spectral energy at 510 nm (μw cm^{-2})
·1
·2
·3
·4
1
2
3
Log rate of change of spectral energy at 510 nm (μw cm^{-2} sec^{-1})

Spectral energy ratios also show seasonal and daily changes. For example, the FR/R (far-red/red; 730/600 nm) energy ratios shown in Figure 7 display significant seasonal patterns of change and occurrence relative to sunrise and sunset.

The air-water interface establishes several important features of the underwater illumination (Smith 1969). Firstly, the 180° aerial horizon is reduced to 97.6°. This causes the sun to rise and set at an apparent zenith distance of 48.5°, instead of 90°. Secondly, incident light rapidly becomes less directional as it penetrates the water. Thirdly, the sun's disc is altered from a point source to a dispersion pattern whose angular composition varies with alt During most of the day the underwater light distribution patte directional, being dependent on the altitude of the sun and its [illegible]ith position. This directional distribution of light is reduced by the presence of clouds. At dawn and dusk there is no longer a distinct area of highest intensity at horizon, but rather a brighter area associated with the zenith. The uniformity of this pattern increases as twilight deepens until a homogenous light distribution is reached. This homogeneity is augmented by the presence of clouds.

In the North Saskatchewan River there is a shift in the spectrum of light towards maximal transmission at shorter wavelengths (500-600 nm) and a reduction in the total and relative spectral energy with increasing depth. Extinction is not the same for all spectral components. However, the overall spectral distribution does become more monochromatic than that of the incident surface light (Fig. 8).

ig. 7. Far red/red (FR/R 730/600 nm) spectral intensity ratios at different times before and after sunrise and sunset. Civil twilight exists approximately during the 40-60 minutes before and after sunrise and sunset (see Fig. 3). Each point is based on the average of 5 ratio values.

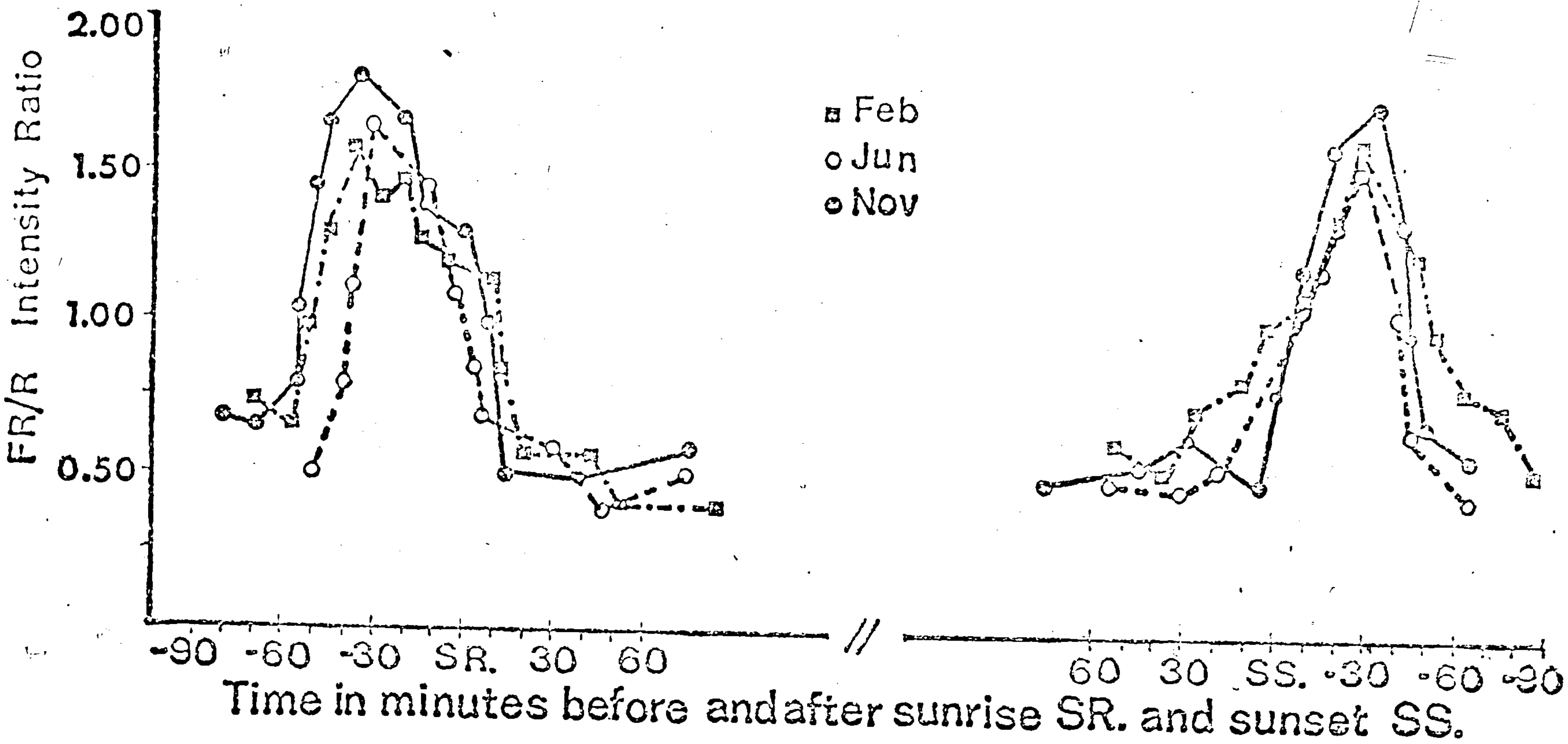
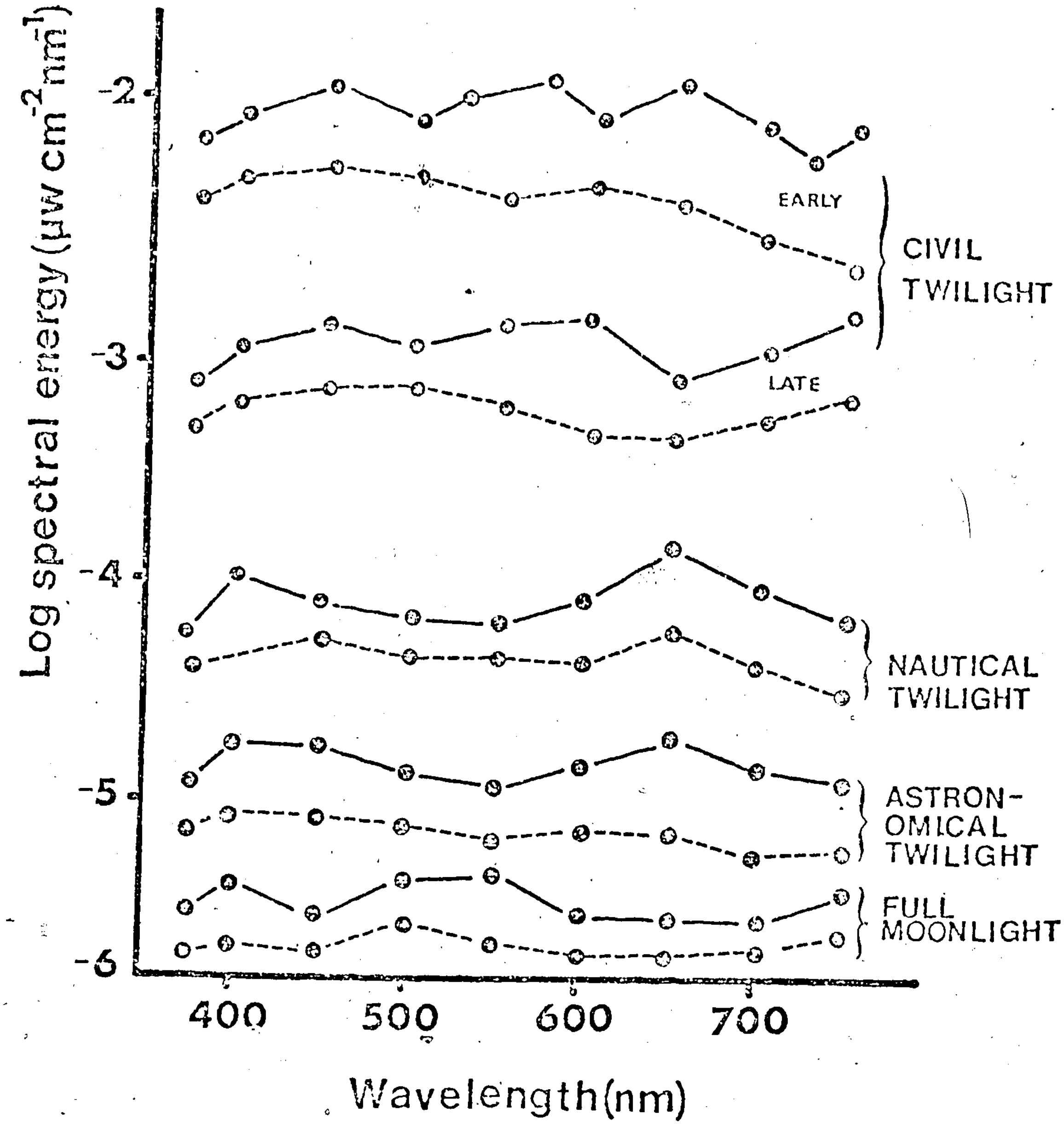

2.00
1.50
1.00
0.50
FR/R Intensity Ratio
■ Feb
○ Jun
● Nov
-90
-60
-30
SR.
30
60
//
60
30
SS.
-30
-60
-90
Time in minutes before and after sunrise SR. and sunset SS.

Fig. 8. Example of spectral energy distributions ($\mu w\ cm^{-2}\ nm^{-1}$) found during civil (early 20 minutes, late 40 minutes to sunrise, respectively), nautical, astronomical twilights and full moonlight at the N. Saskatchewan River. ——— represents surface readings; ----- represents readings from 10 cm below the surface. Values are dawn August 7, 1975.


Log spectral energy (μw cm^{-2}nm^{-1})
-2
-3
-4
-5
-6
400
500
600
700
Wavelength(nm)
EARLY
LATE
CIVIL TWILIGHT
NAUTICAL TWILIGHT
ASTRON-OMICAL TWILIGHT
FULL MOONLIGHT

During twilight the relative reduction in yellow-orange spectral components is not as pronounced as that for surface illumination. Spectral intensity changes, their rates, durations, as well as overall spectral alterations follow the surface solar radiation modifications (see Fig. 8). Seasonal alterations in spectral ratios and rates of change in spectral energy are the same in clear shallow waters as the values that are found at the surface. The similarity to the incident surface spectral alterations and composition permitted initial replications of the underwater spectral alterations in twilight to be based on the more precise and readily obtainable surface irradiance measurements.

The properties of underwater light can be modified by the following limnological factors (Wetzel 1975): 1) water depth, 2) turbidity and water colour, 3) bottom composition and reflectance (albedo), 4) wave action, 5) flow rate and flow velocity, 6) water temperature, 7) primary production, and 8) ice cover.

There is a reduction in spectral energy and transmission with depth. The maximal transmission spectrum shifts to green-light (Munz and McFarland 1975). The exact transmission spectrum is dependent upon the dissolved and/or particulate material present. The dissolved particulate material, particularly phytoplankton, absorbs blue and green wavelengths and can reduce the relative yellow-orange spectral minima seen in the surface twilight spectra.

Diurnal and seasonal variations also exist in silting and can contribute to alterations of light transmission, though other stochastic environmental fluctuations can overrule the more regular effects (Appendix I). In this study the effects of water quality and particulate material were minimized by using clear water for all experimental duplications and taking light measurements from relatively turbid-free shoal regions of the river. Actual spectral intensities found in twilights can vary but the rates of energy change and ratio components remain relatively unaltered.

At shallow depths both incident and reflected light (albedo) have to be considered in determining light levels. Use of the cosine collector incorporates components from both sources but still leaves some error. At the collecting areas the river bottom was fairly uniform, maintaining a constant reflected light level. Thus all recorded light changes could be directly related to surface illumination alterations.

Ice formation reduced spectral intensity and transmission of light. Reliable light measurements could not be made from the portions of the river that were frozen. During ice formation there was a shift towards transmission of longer wavelengths that was accentuated by the presence of surface snow. At the beginning of ice formation, intensity reductions were not significant since there was a simultaneous freezing from the river bottom and consequent increase in reflected light. At spring break-up there is a very dramatic and rapid shift in illumination intensity that can be compared to the initiation of a prolonged pulse of light.

C. Laboratory Light Duplications

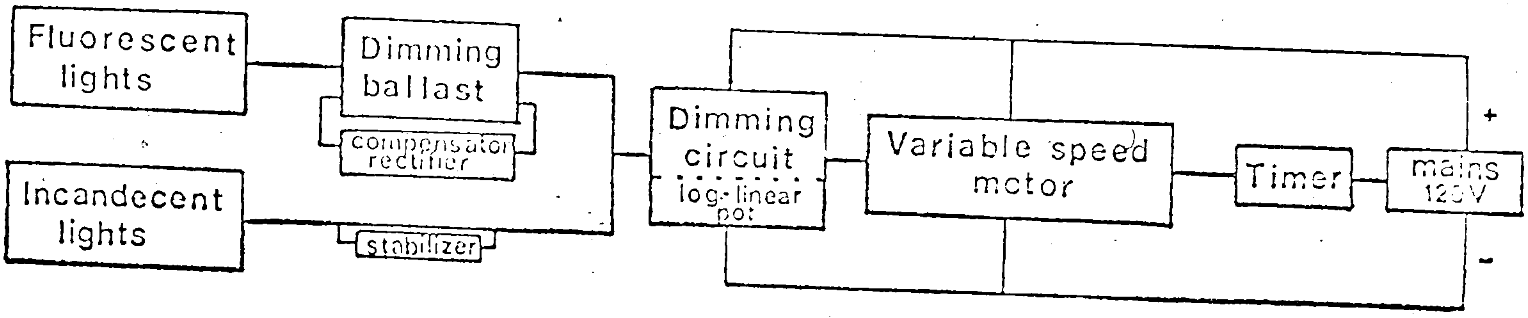
The lighting system used in this study duplicated natural twilight conditions (see Fig. 9). The system was based on features described by Kavanau (1962, 1966, 1968) and Wever (1967) in their 'dawn-dusk machines'. Neither Kavanau or Wever were concerned with exact seasonal duplications of twilight. Only approximate replications of natural twilight durations and occasionally the rates of change of intensity were carried [illegible] by them. For instance, they reported all light intensities in photometric units, ignoring spectral changes that occurred during dimming. However, with slight modifications to their systems it was possible to produce exact replications of intensity and spectral changes occurring in twilights.

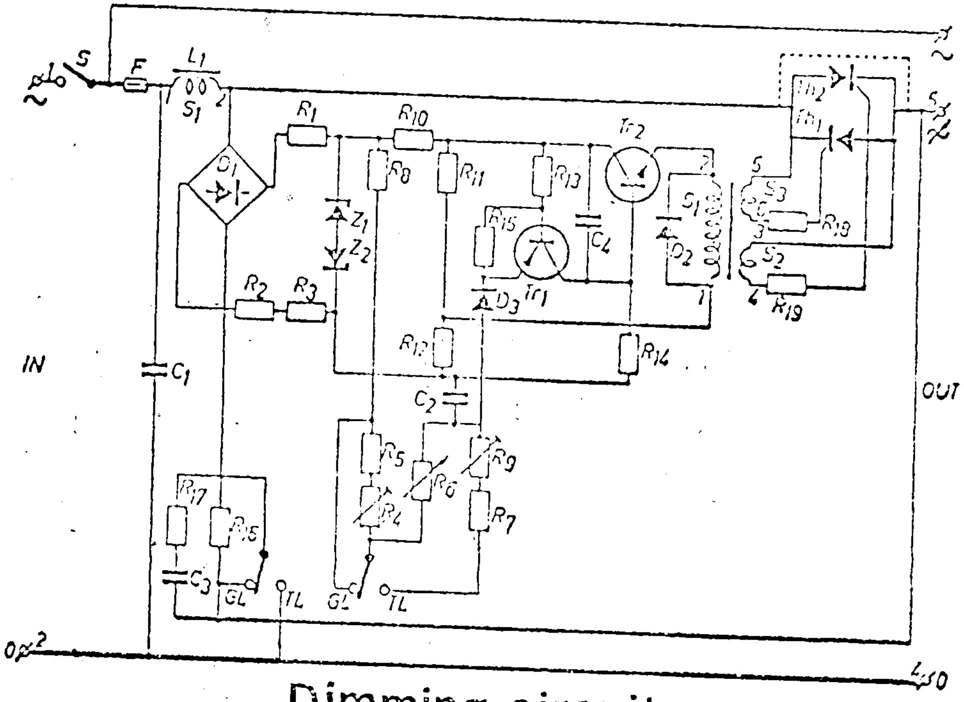
The system used in this study consisted of the following major components: 2 day-lite or vita-lite fluorescent lights (100 and 150 Watt; Sylvania Electric, Toronto); 2 incandescent lights (30 Watt Sylvania) (used only after 'burning in' period); a dimming ballast and associated circuitry to control lamp intensity; and a logarithmetic-linear potentiometer driven by a variable speed motor (Fig. 9). The lighting system was placed over an enclosed plexiglass tank (60 x 90 x 30 cm) and shielded from external disturbances.

By judicious adjustments the rates of change in intensity and spectral composition could closely approximate the natural twilight conditions (see Fig. 9). Seasonal alterations in the rates of intensity change could be corrected by adjusting the log-linear ratios of the potentiometer-motor combination.

As dimming occurs the spectral composition of the fluorescent lamps shift, closely approximating changes found during dawn and dusk. The addition of incandescent lights duplicates far red/red changes and to a lesser degree other portions of the spectrum. Placement of Kodax

Fig. 9. Diagram of apparatus used to duplicate twilights. (Electronic components are listed in Appendix V.)





Dimming circuit

Wratten filters (No. 7 Blue, No. 3 Green; Kodak, Toronto) in front of the lights provided an additional means of manipulating spectral output. However, all of the seasonally occurring combinations of surface spectral components could not be duplicated with equal reliability. Any discrepancies present were determined by continuous recording of light and line voltage outputs.

Underwater spectral changes were a very close approximation of the natural situation. Some of the surface inconsistencies were eliminated by the underwater compression of the spectra. Shifts in the altitude of the sun could not be duplicated. However, the uniform illumination pattern, both at the surface and underwater, permitted the duplicated light to be considered as representative of overcast days (Fig. 10).

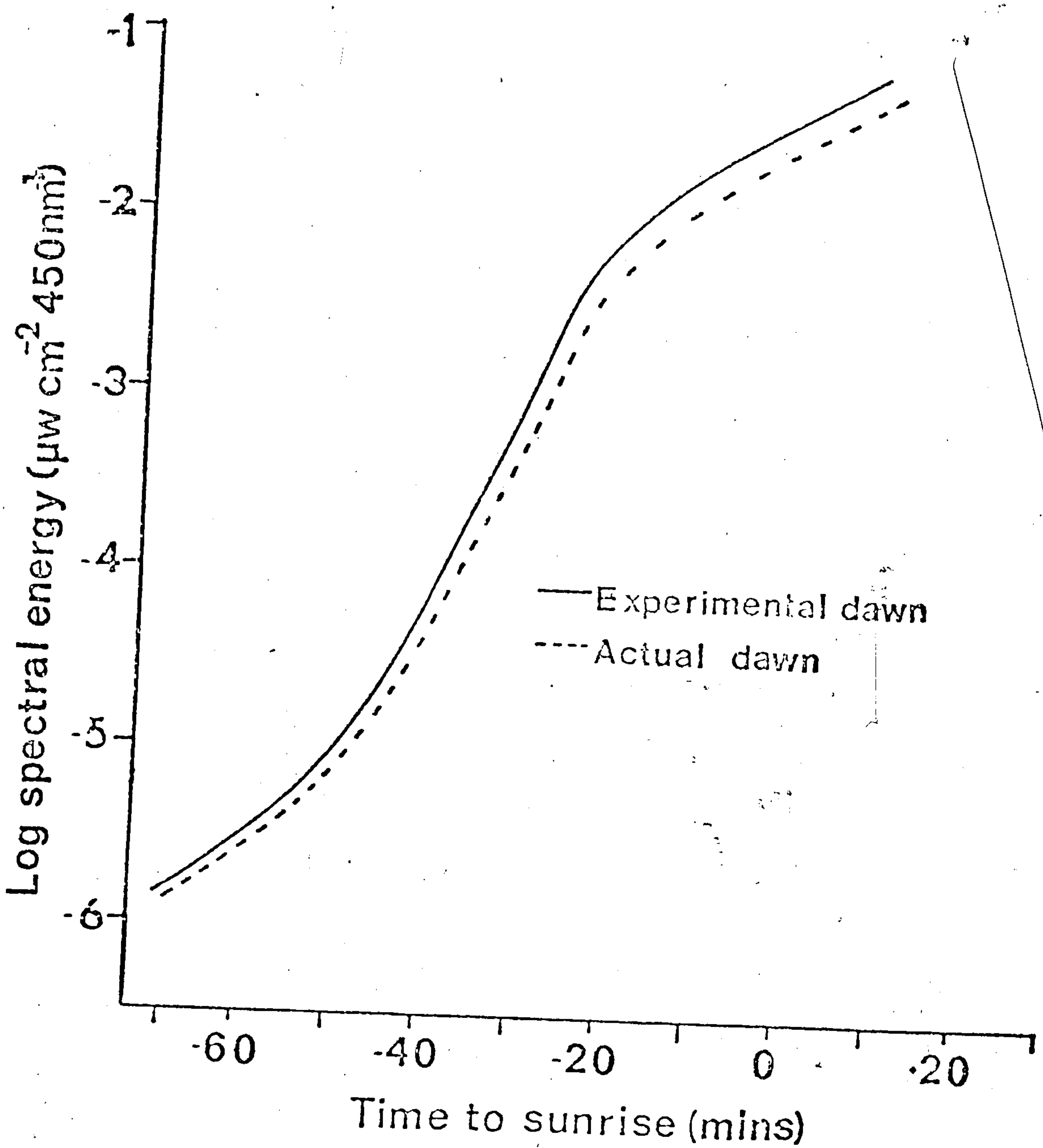
Photoperiods with twilights are represented by, and abbreviated to, LD+t. On-off laboratory photoperiods without twilights are represented by LD. The light-dark transitions are set either to sunrise-sunset or civil twilights. The same settings were maintained within a set of experiments. Light measurements taken from different portions of the experimental tanks indicated that illumination was evenly distributed under LD+t and LD lighting conditions.

D. Methods of Activity Determination

Locomotor activity of *C. plumbeus* was determined by several different means: 1) direct visual observation, 2) photographic/film records, 3) ultrasonic, and 4) electropotential detection methods.

Visual and photographic observations of the activity and behaviour of *Couesius plumbeus* were made at both random and select

Fig. 10. Underwater (10 cm depth) energy values for 450 nm (μw.cm^{-2}) in experimentally duplicated twilights (——) and actual twilights (---). The latter are for an overcast dawn on June 12, 1975.


Log spectral energy (μw cm^{-2} 450nm^{-1})
-1
-2
-3
-4
-5
-6
-60
-40
-20
0
20
Time to sunrise (mins)
Experimental dawn
Actual dawn

30-60 minute intervals. Observations were carried out with fish held in outdoor tanks under natural sky lighting as well as with individuals in laboratory tanks under LD+t and LD photoperiods. Filming of activity was confined primarily to observation of the structure of schools of fish and verification of the accuracy of the electronic methods. Since photographic techniques proved to be unreliable under low or changing light levels, they were not used for any extended observations.

Ultrasonic methods of detecting activity have been used previously with fish (Cummings 1963, Meffert 1968, Byrne 1971). However, these earlier applications were restricted to either (i) detection of the movement of fish past a fixed reference point, or (ii) the recording of unspecified activity of large fishes for limited periods of time. The methods used and described in this thesis permit the detection of movements of fish in all portions of the experimental tank. The ultrasonic systems all function on the same basic principle, that is, detection of alterations in a standing wave pattern of ultrasound (Cummings 1963).

A piezoelectric, ultrasonic transducing element, 2 cm in diameter (Massa Model TR-t, Hingham, Mass.) was attached with silicone grease to each outside end of the tank (Fig. 11). The associated circuit (Fig. 12) was mounted on rubber blocks to reduce vibration and was shielded by a Faraday cage to minimize electrical disturbances. The operating voltage, 6V at 250 ma, was drawn from a stabilized power supply. Positive and negative outputs from the circuit were connected to a chart or an event recorder. The negative lead was connected through a variable resistance (0-100 Ω) acting as a fine sensitivity

Fig. 11. Diagram of apparatus used for activity determinations.

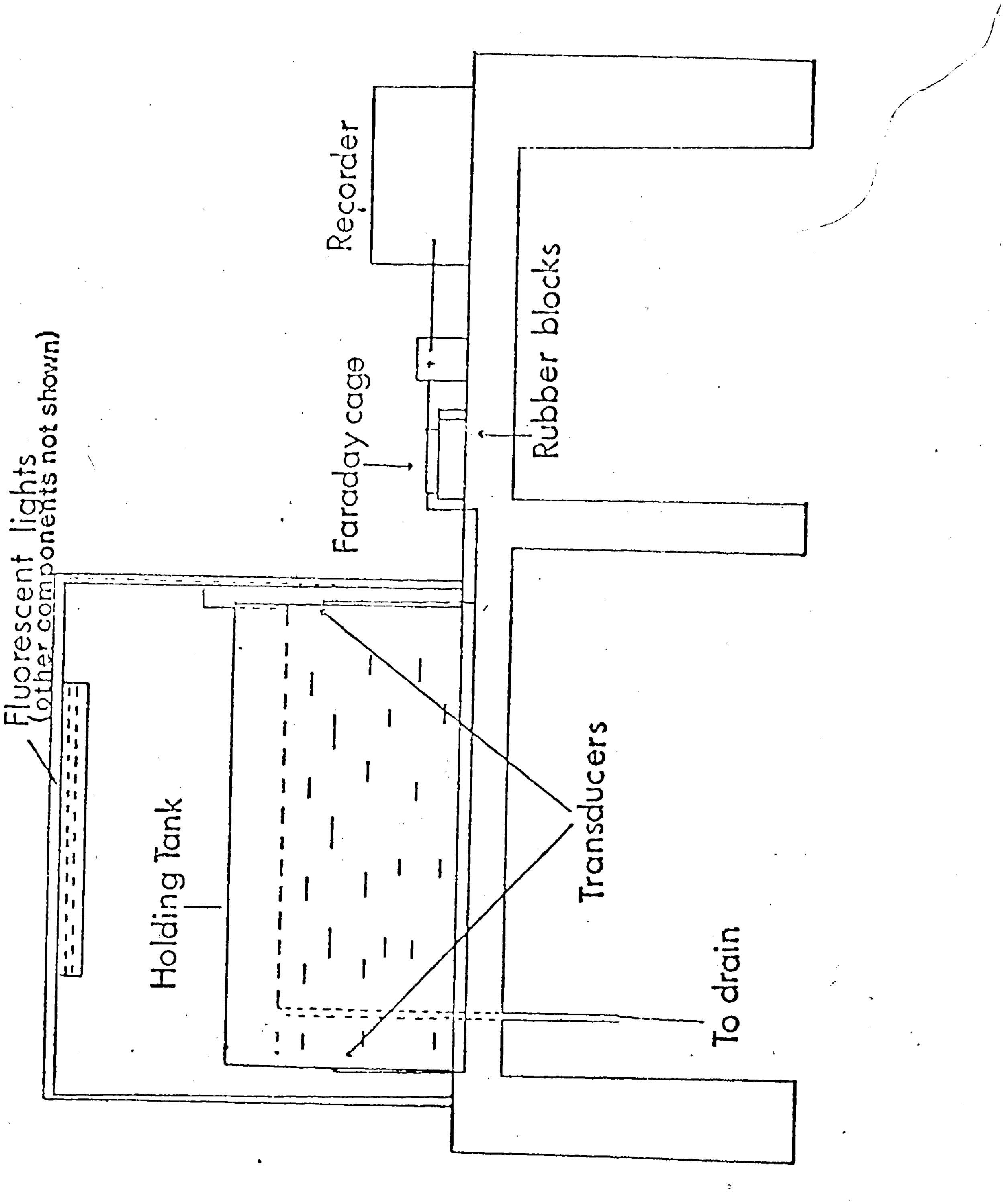
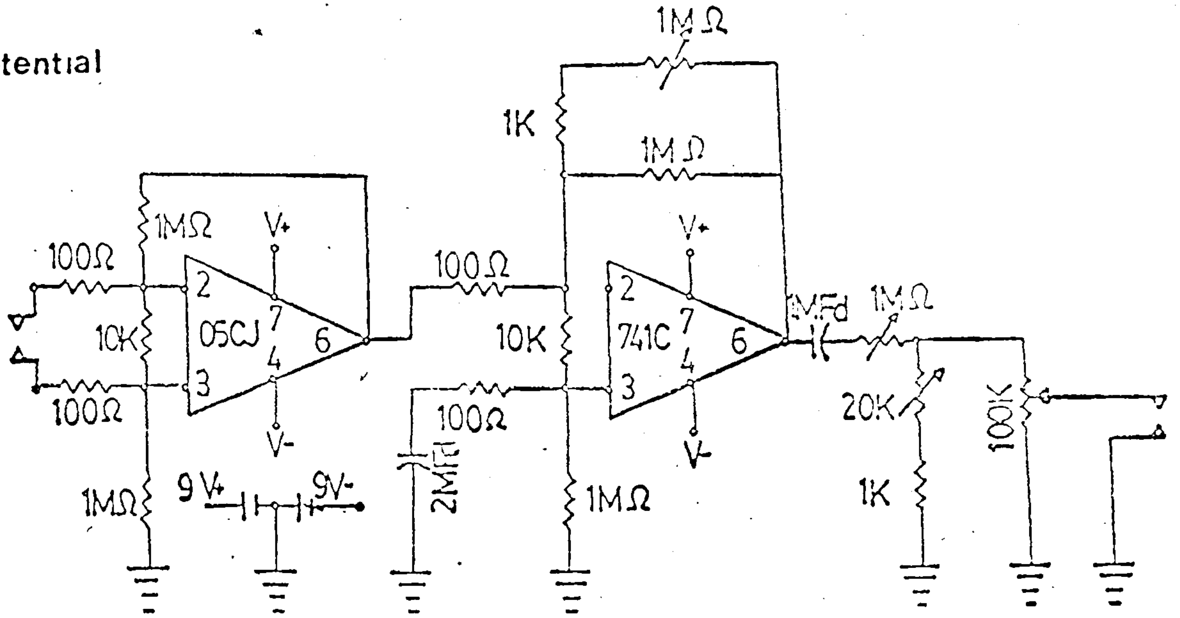
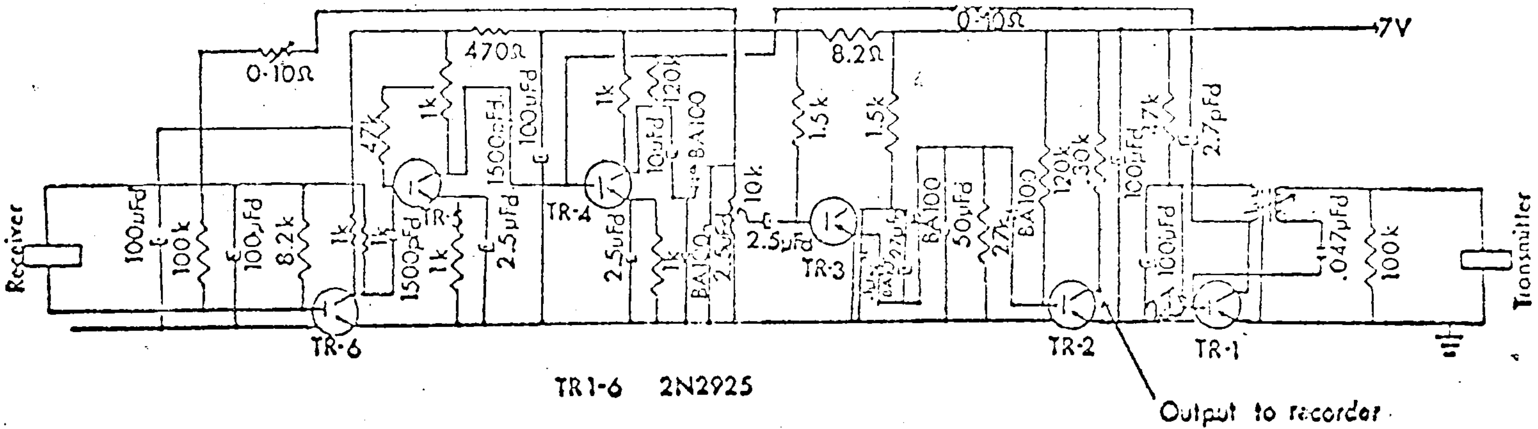

Fluorescent lights
(other components not shown)
Holding Tank
Faraday cage
Recorder
Rubber blocks
Transducers
To drain

Fig. 12. Circuit diagrams of electropotential and ultrasonic detection systems. The electropotential circuit is adapted from Drummond and Davis (1974).

Electropotential circuit



Ultrasonic circuit



control, and a capacitor (400 μFd) functioning as an integrator. Some integration of responses was necessary when the transducer responded more rapidly than the recorder. The tanks, Faraday cage, power supply, and recorder were all connected to a common ground.

One piezoelectric transducer served as transmitter of ultrasound, at an operating frequency of 35 KHz, while the other element functioned as a receiver. The operating frequency of the transmitter could be adjusted from 15-50 KHz by the tuneable coil (Phillips potcore No. 237-A, Amsterdam). Low energy, high frequency, ultrasound has no reported or detectable effect on the activity or behaviour of fishes. The operating frequency of 35 KHz is well above the upper detection threshold of fishes of 3-5 KHz (Tavaloga 1971).

The transmitter set up a standing wave pattern of ultrasound in the tank. A portion of this pattern was detected by the receiving element and transformed into a recordable voltage output, at approximately 100 mV. With a fish in the tank the wave pattern and voltage output were constant as long as the fish remained stationary. By decreasing the sensitivity through an increase in resistance, responsiveness of the apparatus to respiratory gill and postural movements could be eliminated and only gross overall activity would be recorded. With a reduction of detection sensitivity disturbances caused by diffuse water exchange and aeration in the tank did not introduce significant (less than 20 mV) fluctuations on the chart records.

Resistance was adjusted such that when a fish moved both the phase and frequency (by Doppler shift) of the standing wave pattern changed, altering the received pattern. These alterations were measured as a change in the voltage level by 20 to 100 mV and represented by a peak on the chart recording (Figs. 13 and 14). More detailed descriptions of the Doppler shift principle are provided by Meffert (1965). Calibrations, controls, and interpretations of the activity records will be discussed after operation of the electropotential method is considered.

The electropotential method of detecting activity also involved the recording of voltage changes caused by the locomotory motion of fish. These alterations were induced by the movement of a fish in the tank altering a naturally occurring electric potential (50 to 200 mV). The exact detection and operating techniques are described by Spoor et al. (1971).

A stainless steel electrode, 10 cm in length, was placed at each end of the tank. These electrodes measured an equilibrium potential that was then recorded and transformed to approximately 100 mV. When a fish moved this potential was altered by 20 to 100 mV, and a peak was obtained on the chart recording. A balancing circuit and amplifier (Drummond and Dawson 1974, and see Fig. 12) were used to adjust the sensitivity and obtain unidirectional positive voltage shifts. A capacitor (400 μFd) acted as an integrator to compensate for recorder response time. The sensitivity of the circuit could be adjusted so that gill, respiratory, postural movements, along with aeration and flow of water into the tank were not detected.

The electropotential method, which is technically simpler than the ultrasonic procedure, was used more extensively for the detection

Fig. 13. Examples of locomotor activity of *C. plumbeus* obtained with ultrasonic techniques and recorded at a high chart speed (0.025 cm sec^{-1}). Water temperature 12°C; 12:12 LD; L 125 μw cm^{-2} (350-750 nm). Similar results were obtained with the electropotential techniques (see Fig. 14).

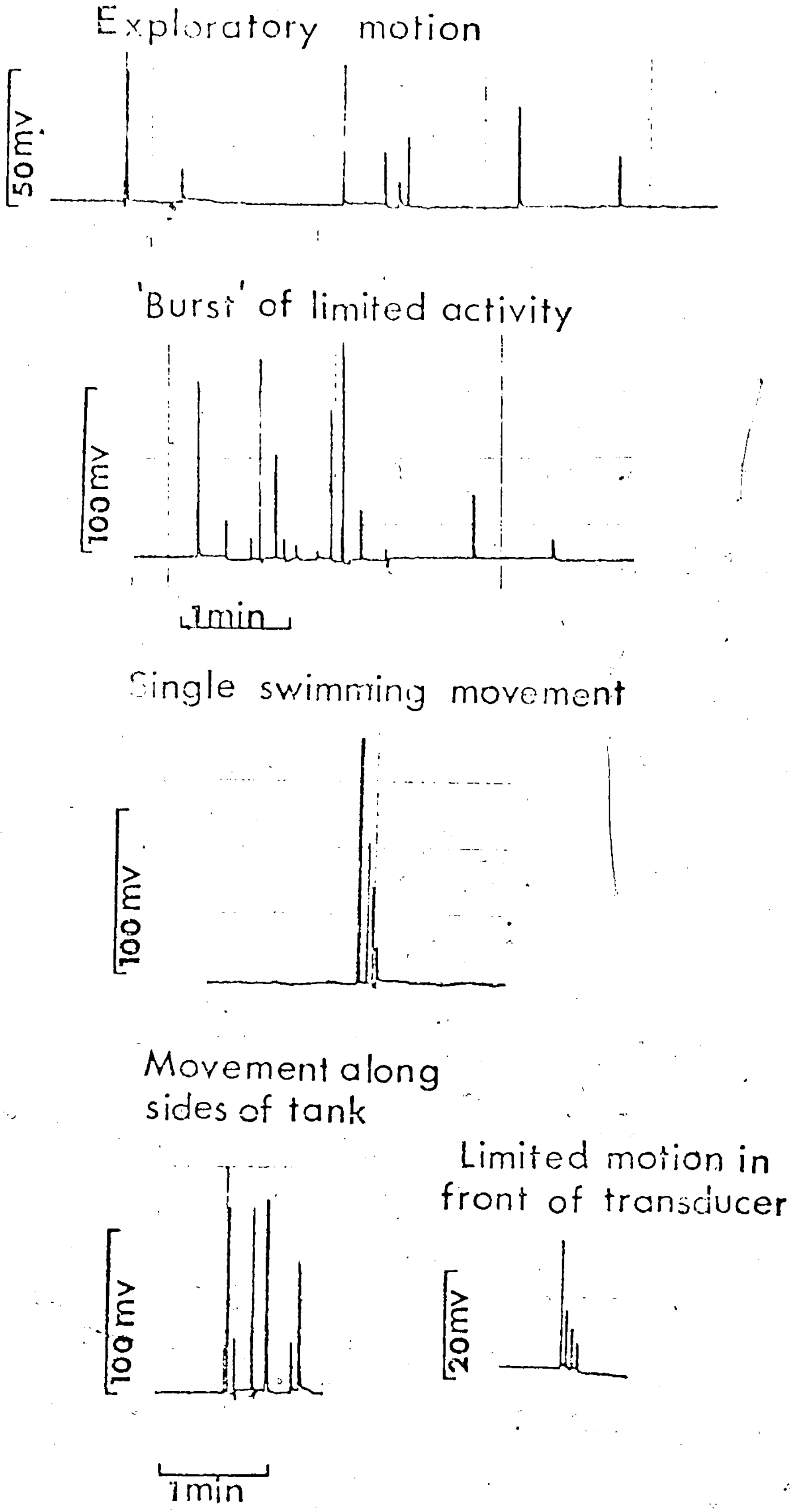

Exploratory motion
50mv
'Burst' of limited activity
100mv
1min
Single swimming movement
100mv
Movement along sides of tank
100mv
1min
Limited motion in front of transducer
20mv

Fig. 14. A Examples of various locomotory behaviours of *C. plumbeus* recorded with the electropotential detection system (6°C; 9:15 LD).

B Examples of various locomotory behaviours of *C. plumbeus* recorded with the electropotential detection system (12°C; 15:9 LD).

C Examples of various locomotory behaviours of *C. plumbeus* with the ultrasonic detection system (8°C; 12:12 LD).

(Cont'd)

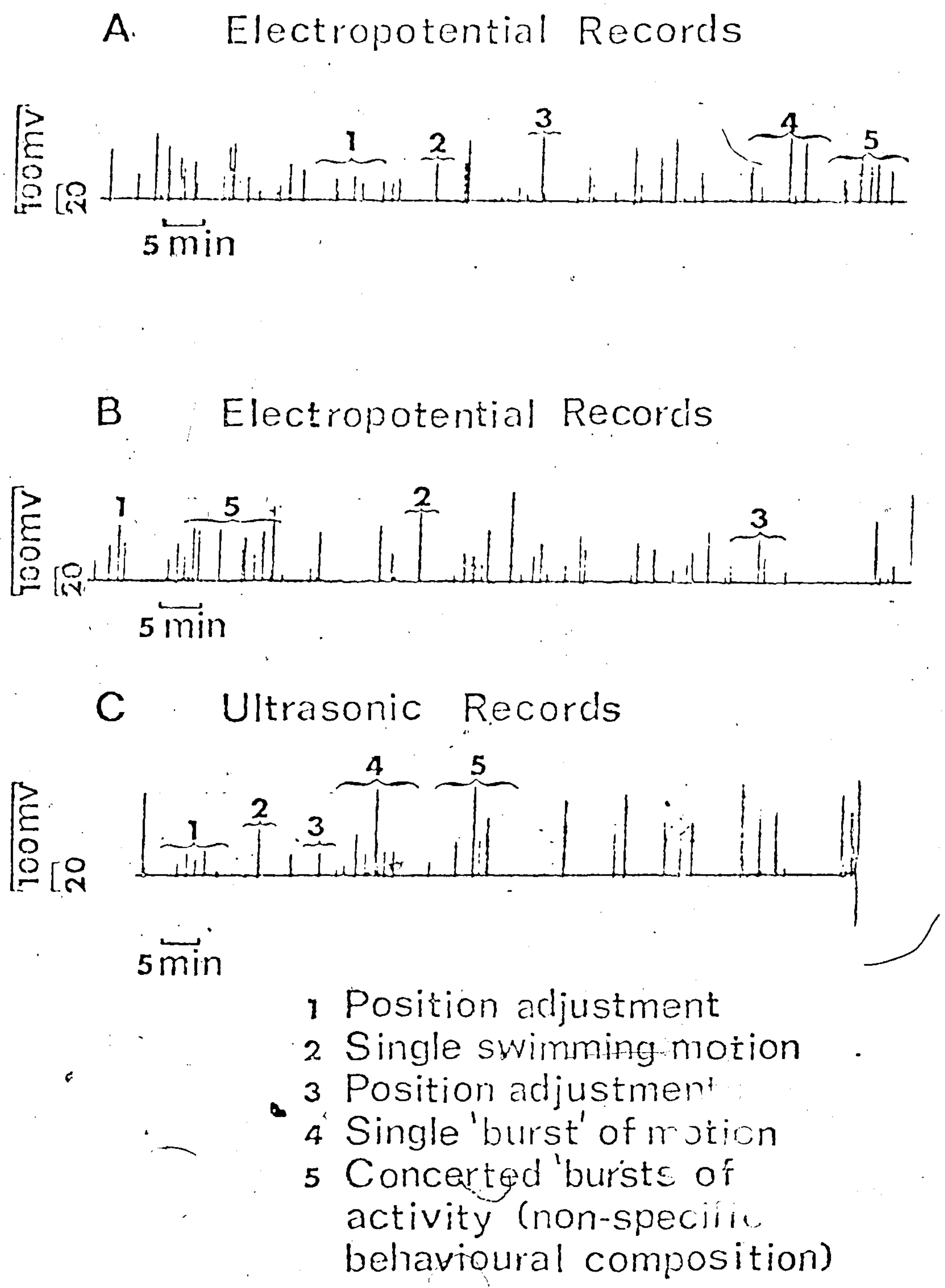
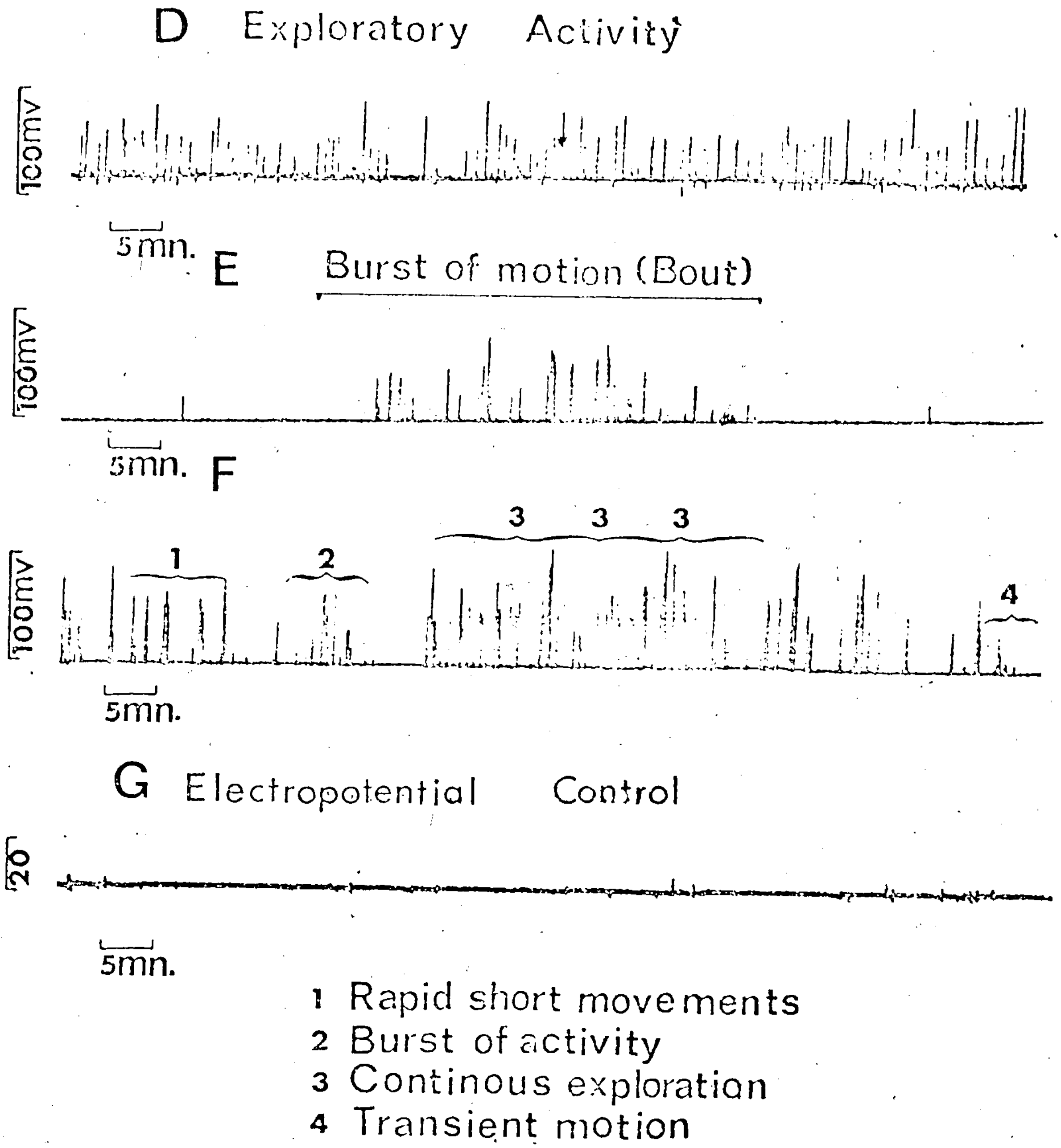

A Electropotential Records
100mV
20
1
2
3
4
5
5 min
B Electropotential Records
100mV
20
1
5
2
3
5 min
C Ultrasonic Records
100mV
20
1
2
3
4
5
5 min
1 Position adjustment
2 Single ~~swimming~~ motion
3 Position adjustment
4 Single 'burst' of motion
5 Concerted 'bursts of activity (non-specific behavioural composition)

Fig. 14. D-F Examples of various locomotory behaviours of *C. plumbeus* recorded with the electropotential detection system (10°C; 12:12 LD).

G Example of control electropotential record obtained with no fish but with aeration in the experimental tank (10°C).


D Exploratory Activity
100mV
5mn.
E Burst of motion (Bout)
100mV
5mn.
F
1
2
3 3 3
4
100mV
5mn.
G Electropotential Control
20
5mn.
1 Rapid short movements
2 Burst of activity
3 Continous exploration
4 Transient motion

of activity. However, as will be shown, the voltage and chart results/records obtained by the two methods were qualitatively and quantitatively the same. In the results presented no distinctions are made as to which method was used to record the activity of *C. plumbeus*.

The height and number of peaks recorded by both detection methods were approximately proportional to the degree and total amount of activity, respectively, of the fish (see Figs. 13 and 14). These relationships were established by visual observation and/or filming of fish activity while recording their locomotory motion. The height of the peak was, within the limits of integration, proportional to the strength or extent of the swimming activity. Continuous activity greater than 30 seconds gave a continuous set of peaks, as the phase of the received ultrasound or the electrode potential was constantly changing (Fig. 14). Highly concerted bursts of motion resulted in a summation or integration of a number of rapid (less than 1 sec duration) consecutive voltage outputs into a single larger discharge value. After a single swimming motion or 'burst of activity' there were several transient voltage peaks (less than 20 mV) the exact number depending on the sensitivity of the detector (see Fig. 13). These smaller peaks were discounted as sources of activity. However, these transient peaks and overall variations in voltage levels prohibited the use of event recorders such as the Esterline-Angus, that are used with studies of the locomotory activity of birds and mammals. Event recorders indicate either a maximal response or no response. They do not permit the determination of gradients or discontinuities in activity.

Determinations of the sensitivity of the detector systems were achieved by (i) observing the motion of both the same and different fish and noting activity and corresponding chart records, and (ii) artificially displacing water at different portions of the tank and noting chart responses. The results obtained by the two electronic methods were virtually equivalent (Table 1). The placement of electrodes inside the tank did not significantly affect or alter the laboratory behaviour of *C. plumbeus*. The magnitude of the peaks or voltage values, total number of peaks recorded for equivalent movements or disturbances, were not significantly different between the two methods (Mann-Whitney U test $p > 0.50$). Sensitivity could be adjusted to obtain equivalent results with different water temperatures.

Locomotory activity could be detected at all portions of the tanks, with maximal sensitivity being along the sides of the tanks and minimal sensitivity on the plane connecting the transducers or electrodes. These disparities in sensitivity were minimized by adjusting the relative positions of the two detectors. (However, this reduced information about the exact location of the fish.) The locations of the detectors were determined by trial-and-error procedures and had to be readjusted for significant changes in water characteristics. All determinations of activity were carried out with clear water. Under turbid conditions settling of particles and alterations in suspension characteristics introduced spurious and shifting results.

The outputs of the detecting systems were recorded without any fish in the tank. A single base-line, displaying occasional drift, was obtained (see Fig. 14). There were small voltage disturbances

Table I. Comparison of activity records, expressed as millivolts (mV) for different behaviours of *C. plebeae* as obtained by ultrasonic and electropotential recording techniques.

	Behavioural Category and Voltage Level (mV) (Mean and Range)		
	Position adjustment	Limited movement	Exploratory behaviour
Ultrasonic[1]	29 (22-36)	38 (35-45)	75 (50-100)
Electro-potential[1]	30 (23-37)	41 (34-47)	79 (50-105)

[1] Mann-Whitney U test reveals no significant difference between values obtained by the two recording techniques ($p = 0.40$).

(1-5 mV), but these were below the amplitudes necessary for classifying them as activity. These voltage shifts were primarily the result of electrical disturbances and did not have any effect on the total activity determined. These control measurements were repeated at various times throughout the year.

Activity was continuously recorded at relatively high chart speeds that ranged from 0.25 to 0.0025 cm sec^{-1}. For analysis, peak totals were summated over 1 to 30 minute intervals. The time interval used depended upon the factor(s) being investigated. This procedure reduced the unwieldiness of the records and permitted computer analyses of activity time series. Only peaks that met the criteria previously described were used in the computation of activity totals. (To simplify graphical presentations, activity totals were transformed into proportional, relative, or arbitrary units.)

For equivalent behaviours and degrees of motion/activity, the activity totals obtained by the electropotential and ultrasonic techniques were the same (Figs. 13, 14 and Table 2; Mann-Whitney U test $p > 0.5$). Therefore no distinction has been made between ultrasonically or electropotentially recorded activity in the subsequent analyses.

The use of activity totals gives a partially distorted representation of locomotory behaviour. The activity of *C. plumbeus* occurred in bursts of several seconds to minutes duration (see Fig. 14) that were concerted into longer bouts. (A bout is defined as a period or portion of activity that has no inactive 'rest' periods greater than 5 minutes duration.) However, since graphical representations were based on

Table 2. Comparisons of activity totals (10 minute) for different behaviours of *C. plumbeus* as obtained by ultrasonic and electropotential recording techniques.

	Behavioural Category (Mean and Range)		
	Position adjustment	Limited movement	Exploratory behaviour
Ultrasonic[1,2]	7 (4-18)	13 (11-16)	28 (21-39)
Electro-potential[1,2]	6 (4-[illegible]	14 (10-17)	26 (19-41)

---

[1] Values for each [illegible]our are based on a sample of 100 (10 different intervals from 10 different individual *C. plumbeus*.

[2] Mann-Whitney U test reveals no significant difference between activity totals obtained by the two recording techniques (p = 0.50).

activity totals, this discontinuous nature was not always readily apparent. The rapid (30 sec-3 min) discontinuities were determined by visual observation, while larger scale alterations were obtained from computer analyses.

With practice from concurrent observations of activity and chart records, the behaviours recorded could be determined. However, all determinations of behavioural composition were obtained from visual and film observations. Comparisons with chart records were used solely as a check on the accuracy of the detecting system.

E. General Experimental Procedures

Locomotor activity and behaviour patterns of single *C. plumbeus* were observed and recorded over an annual cycle of photoperiod and twilight. Experiments were repeated for 1974, 1975, and 1976. Twelve fish were used for each of the annual analyses. Actual experimental procedures were conducted with a greater number of fish. These latter individuals were used, however, for additional experiments (Appendix II). Comparisons were made between the activity of freshly captured fish obtained at the sampling intervals and fish that had been kept under laboratory photoperiod conditions (LD, LD+t). The exact experimental procedures followed for each of the fish investigated are listed in Appendix II.

Two tanks were placed under the twilight simulator (described on p. 31. One (Tank A) was used for longer term activity recording, while the other (Tank B) was used primarily for fish acclimation. Tank B could be partitioned by white plexiglass baffles into several compartments. After 48-72 hrs of acclimation individuals from Tank B were placed into Tank A and activity was recorded for 4-5 days. Exchanges of fish were conducted at random times of the day throughout the year. These changeovers permitted analyses for age, sex, group size, and environmental differences.

At approximately 1-2 week intervals new samples of fish were obtained. Individuals from these catches served as controls, and were placed under the simulated twilights and their activity was monitored. Additions of new animals or alterations of experimental conditions were not carried out at solstices and equinoxes. At select monthly intervals fish were removed from LD or LD+t and placed under constant conditions (constant dark DD or constant light LL) and their activity was continuously recorded. More specific procedures followed are discussed in the appropriate chapters.

Chapter III

PRELIMINARY RESULTS

According to accepted convention the following two criteria have to be fulfilled in order to demonstrate the presence of an endogenous circadian rhythm (Bünning 1973). Firstly, the rhythmicity must persist for at least 3-7 cycles under constant conditions and have a free-running period (FRP) designated Tau (T) of approximately, but not exactly, 24 hours. Secondly, a 24 hour environmental periodicity, specifically photoperiod or light-dark (LD) cycle, has to be able to entrain, that is, control both the period and phase of the endogenous cycle.

In preliminary analyses single *C. plumbeus* were removed from the holding tank and placed in the experimental tank (A) set at a seasonally appropriate water temperature (Appendix I) and under a 12:12 LD cycle (0.01:150 μw cm^2). Fish were held under these conditions for 5 days after which constant conditions, either constant dark (DD) or constant light (LL), were imposed for 7-10 days. Activity of the fish when under LD and DD or LL was continuously recorded by the electropotential or ultrasonic method. Locomotory behaviour was then plotted as activity totals over 15 minute intervals. These procedures were repeated with 8 different fish.

A sample record of the locomotor activity of a 3 year female *C. plumbeus* held under LD and then DD is given in Figure 15A. Under the LD photoperiod a bimodal diel pattern of activity was evident. With the onset of DD the activity pattern lost synchrony with the

Fig. 15. A Locomotory activity record of a single *Couesius plumbeus* under LD (0.01; 150 μw cm^{-2}; 16°C) for 2 cycles. Constant dark (DD) was then imposed for 10 days and activity was continuously recorded.

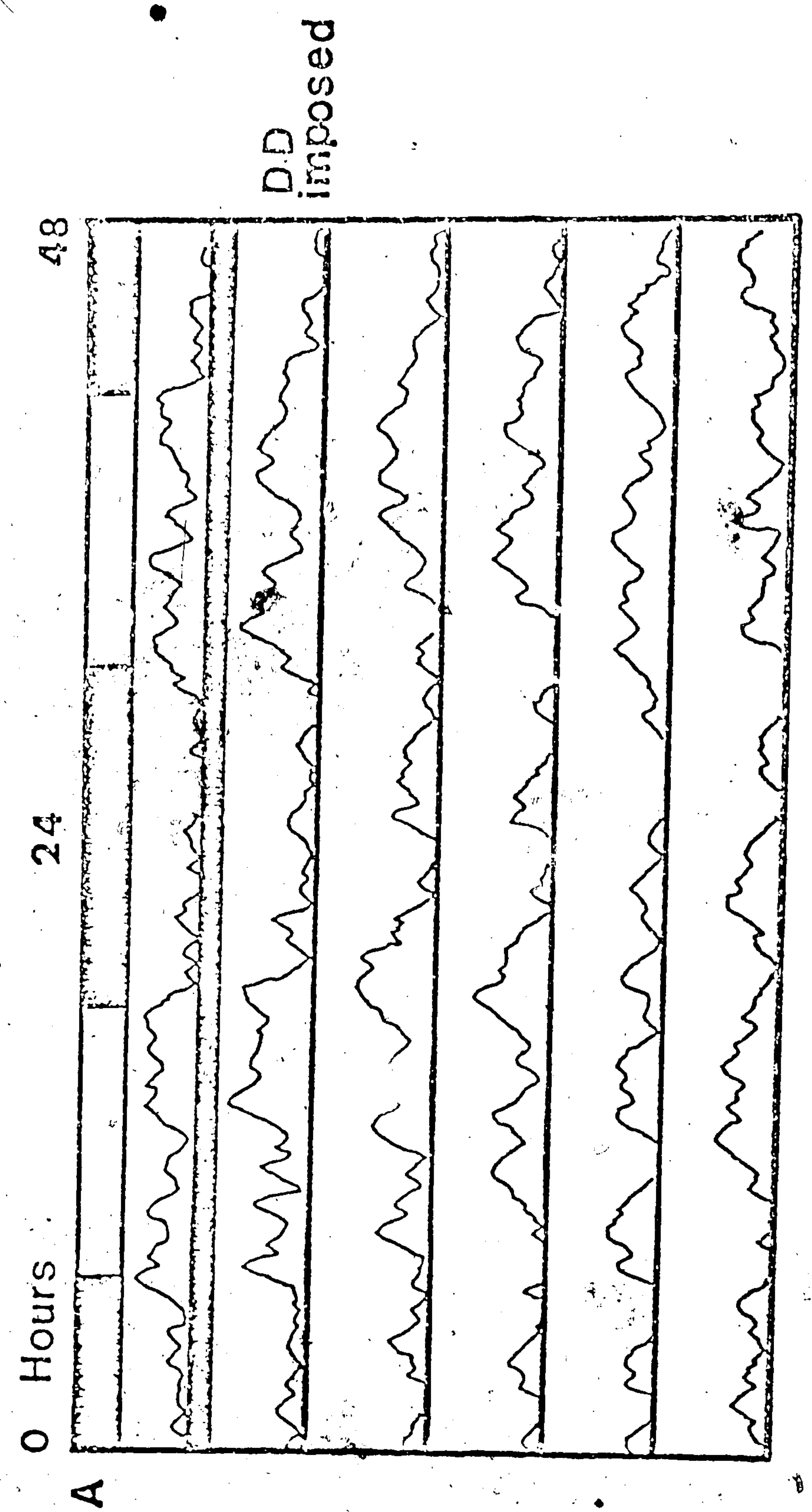
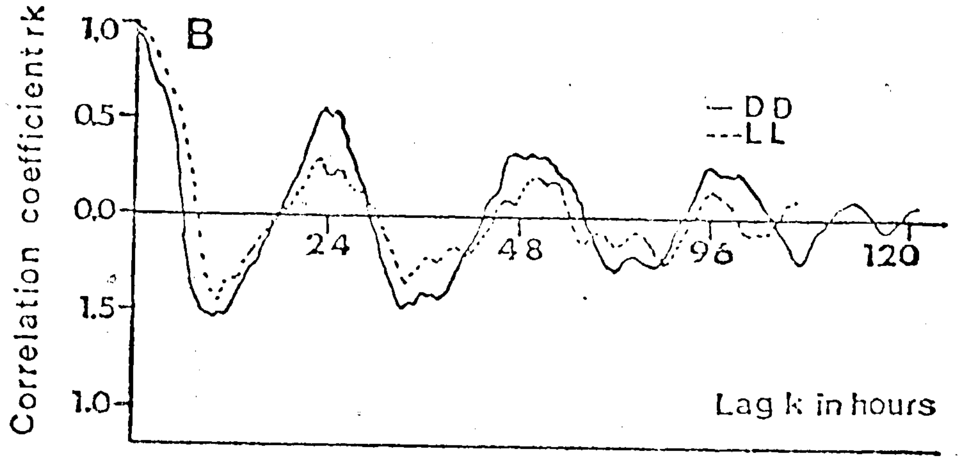
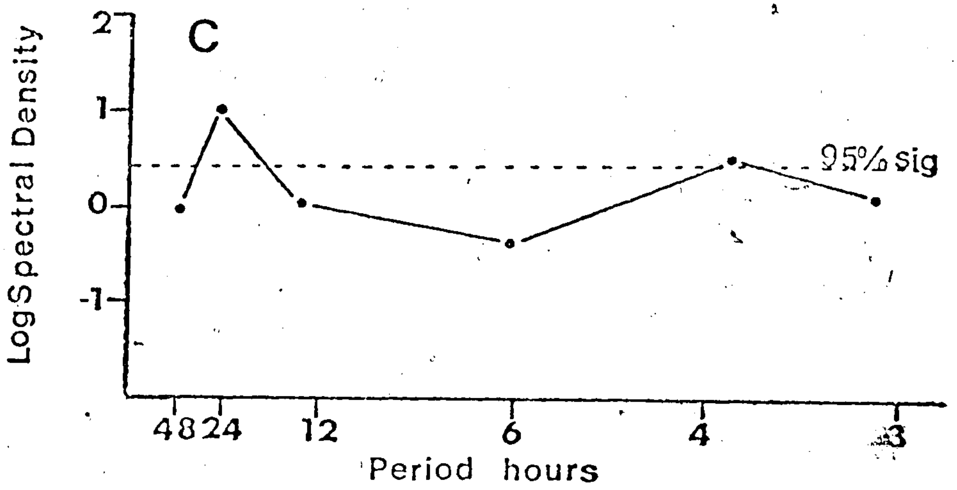

A
0 Hours
24
48
D.D
imposed

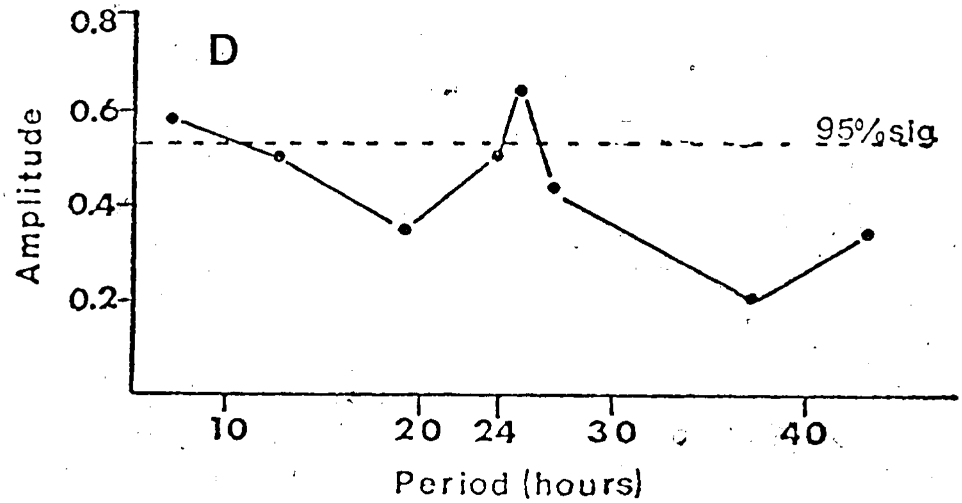
Fig. 15. B Autocorrelation plo [illegible] the previous (DD) record after normalizing, standardizing and detrending.

C Power spectrum transformation of the autocorrelation plot.

D Periodogram analysis of the locomotory record presented in A. The period value is 25.6 ± 0.4 hrs. The horizontal lines in C and D represent the upper 95% significance levels.


B
Correlation coefficient rk
1.0
0.5
0.0
1.5
1.0
— DD
--- LL
24
48
96
120
Lag k in hours


C
Log Spectral Density
2
1
0
-1
95% sig
48
24
12
6
4
3
Period hours


D
Amplitude
0.8
0.6
0.4
0.2
95%sig
10
20
24
30
40
Period (hours)

previous photoperiod and after approximately 6 cycles extinguished to an apparent mean level of non-stationary arrhythmic activity.

Approximate 24 hour rhythmic patterns were evident in the activity plots from DD and LL conditions (the latter are not shown). Estimates of period values could be obtained by determining the times between consecutive maximal activity onsets.

With serially correlated non-stationary data such as activity and behaviour records it is necessary to employ statistical techniques that do not rely on independent data samples (Enright 1965). Time series methods, specifically the periodogram (Enright 1965), and autocorrelation and power spectra techniques (Bendat and Piersol 1968; Dixon 1971), fit these requirements. The periodograms computed amplitude for every possible period value that was within the resolution limits of the sampling interval. A significant period indicated that a particular frequency was present in the activity series. For example, in Figure 15D the periodogram indicates that the activity under DD had a free-running period of 25.6 ±0.4 hrs. All of the fish held under DD or LL evinced free-running rhythms of locomotory activity for at least 4 cycles, the exact duration varying between individuals and constant conditions employed. Preliminary determinations indicated that activity under LL (not shown) extinguished more rapidly than that from DD. Therefore DD conditions were used in the majority of subsequent period analyses. These results show that an endogenous rhythm with a period of approximately 24 hours is present in the activity of *C. plumbeus*. This meets the first criteria for demonstrating the existence of an endogenous rhythm of locomotor activity.

When arrhythmic *C. plumbeus* under DD or LL were exposed to 12:12 LD cycles they displayed a diel activity pattern with a period of approximately 24 hrs (± 0.10 hrs). This strongly suggested, but did not confirm, that photoperiods could synchronize the endogenous rhythm of activity.

In order to conclusively show that photoperiods are entraining activity it is necessary to demonstrate that a phase-shift in the light-dark cycle is accompanied by a corresponding shift in the activity cycle (Bünning 1973). In these preliminary experiments the 12:12 LD cycles (0.01:110 μw cm^{-2}) were phase shifted 90° (6 hrs) or 180° (12 hrs), all relative to the initial LD cycle, and subsequent changes in activity recorded (Fig. 16). This procedure was repeated with 5 different fish. The representative result presented (see Fig. 16) indicates that activity shifted with photoperiods.

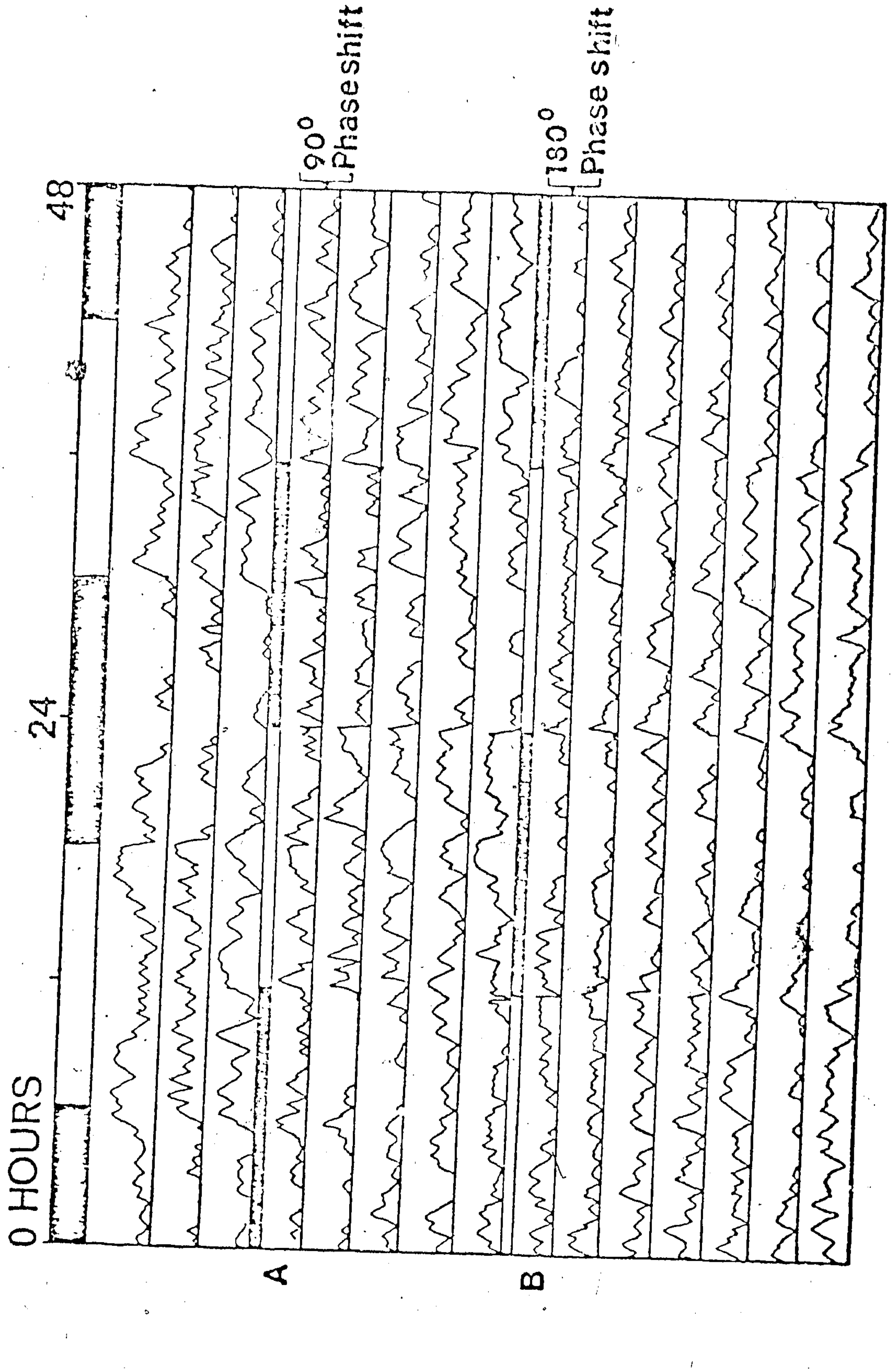
Phase stability and relatively stable phase relationships were gradually obtained after 15-20 cycles, but approximate synchrony was evident after 3-5 cycles. The criteria used to determine entrainment were a relatively stable relationship between the beginning of activity and the onset of L in the photoperiod and the presence of a 24 hour period in activity. In a study of the time course of entrainment Kramm (1975) showed that a continuous oscillation in entrained periods and phase values around steady-state values, in the form of a limit cycle, existed. According to these criteria it is evident that the LD cycle entrained the circadian rhythm of locomotor activity of *C. plumbeus*.

Fig. 16. Locomotory activity of a single *Couesius plumbeus* under a 12:12 LD cycle (0.01:150 μw cm^{-2}) that is phase-shifted

A 90°

B 180°

Activity is in arbitrary units based on half-hour activity totals. Time is in Mountain Standard Time.


0 HOURS
24
48
A
B
90° Phase shift
180° Phase shift

These preliminary experiments demonstrated (i) the existence of a diel rhythm of locomotor activity under LD cycles; (ii) the presence of an endogenous circadian rhythm of locomotor activity; and (iii) that the endogenous rhythm of locomotor activity could be entrained by LD cycles, or daily cycles of illumination.

Chapter IV

GENERAL ACTIVITY AND ENTRAINMENT

A. Behavioural Aspects

(i) Introduction

Responses to exogenous environmental factors, including components of photoperiods, can vary according to the behavioural and physiological state of the animal. Without a knowledge of the behavioural composition of activity a limit is rapidly imposed on the amount of information that can be obtained by an examination of entrainment (Enright 1970, Menaker 1976). Therefore, a preliminary description and analysis of the components of locomotory activity is necessary.

(ii) Methods and results

Quantitative visual observations of a total of 146 single and 20 groups of 8 *C. plumbeus* were carried out at select 30-60 minute intervals. Lake chub from the outdoor tanks and laboratory tanks held under LD+t were used. Observations were carried out throughout the year (1974, 1975, 1976). Winter (Dec-Mar) observations could only be done effectively with individuals under artificial LD+t photoperiods. All observations were carried out with freshly captured individuals as well as with lake chub that had been in the laboratory for more extended periods of time. Since no significant differences were found between the results obtained from outdoor and LD+t conditions (Mann-Whitney U test $p \approx 0.05$), pooled results are presented.

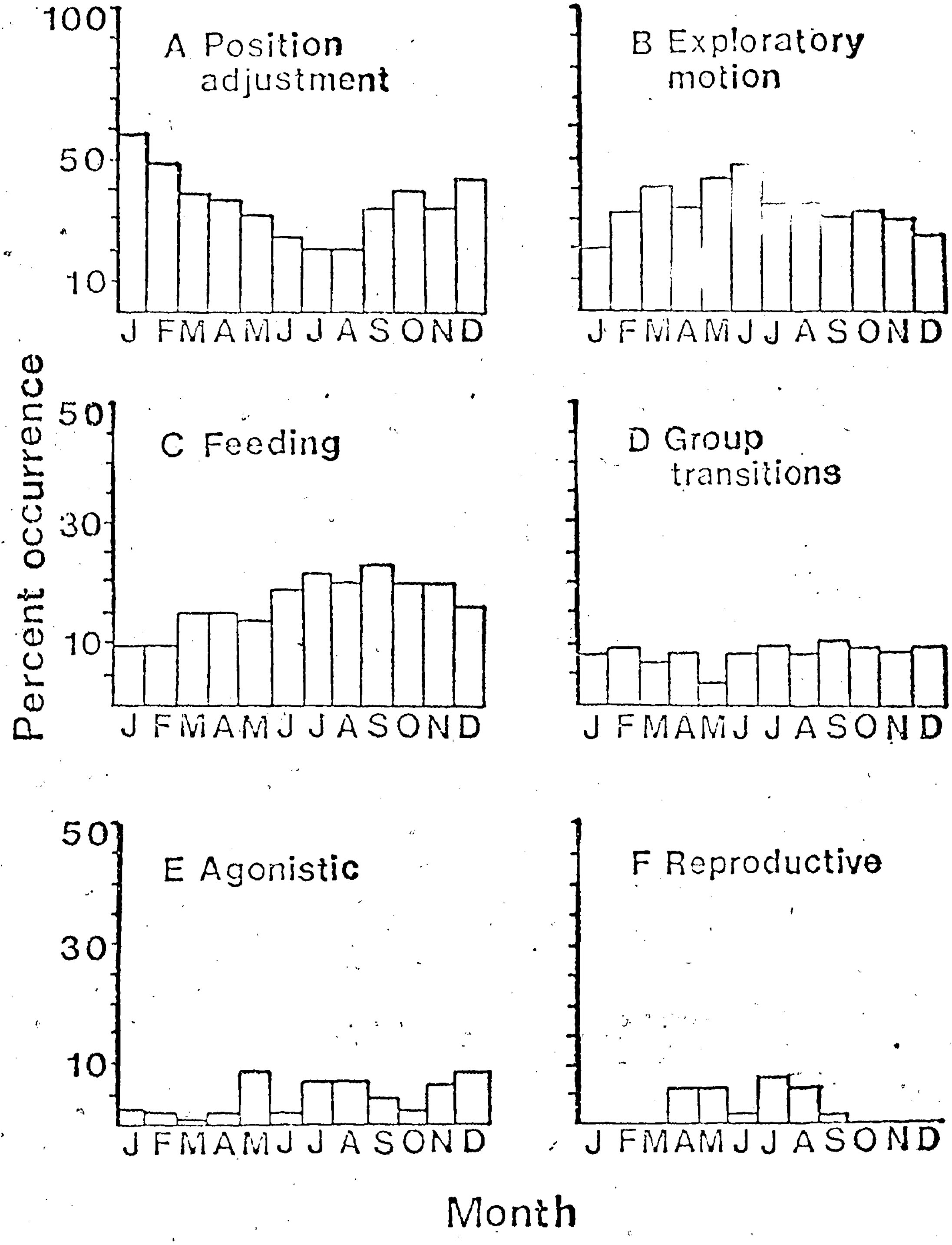
Focal animal sampling techniques, that is, observation of 'key' appetitive or consummatory behavioural components in a single animal, were used to maximize the relative amount of data obtained from observation periods of 30-60 min (Altmann 1974). Film records were used to supplement the visual observations of group behaviour.

The frequency and duration of behaviours were recorded by a key-board and 8-channel event recorder (Esterline-Angus, New York) or by a tape recorder. Behaviour patterns were classified according to their apparent biological function(s). Six general behavioural categories were recognized. These categories included: 1) position adjustment and holding ; 2) inter- and intra-specific agonistic interactions (chasing, nipping, etc.); 3) feeding and associated motions; 4) schooling and transitions between schools, aggregates, and individuals; 5) reproductive and sexually associated behaviours, and 6) exploratory motions.

These behaviours were chosen because they met at least one of the following criteria: they were relatively easy to score; the same general types of behaviours were present in all fish; their proportions varied between different times of the year: and the categories had proven useful in previous studies of fish behaviour (Baerends 1971).

The relative contribution of these 6 behavioural divisions to total diel locomotory activity undergoes seasonal (monthly) fluctuations (Fig. 17). During the longer summer photoperiods (May-Aug, with 10-16 hrs total light) a greater proportion of activity, 22% as compared to 10% (for feeding), is devoted to feeding and exploratory behaviour. Actual reproductively related behaviours have an extremely limited duration, reproduction being highly dependent on water temperature and the presence of a proper physical substrate (see Brown et al.

Fig. 17. Seasonal distribution of the per cent behavioural composition of the locomotory activity of *Couesius plumbeus* relative to total activity composed A) position adjustment and limited motion; B) exploratory motion; C) feeding; D) group transitions (school - aggregate - individual); E) agonistic; F) reproductive-sexual behaviours.


A Position adjustment
B Exploratory motion
C Feeding
D Group transitions
E Agonistic
F Reproductive
100
50
10
50
30
10
50
30
10
J F M A M J J A S O N D
Percent occurrence
Month

1970 for descriptions of the reproductive biology of *C. plumbeus*). The total amount of time devoted to feeding was greater during increasing than decreasing photoperiods of equivalent length. Inter- and intra-specific interactions varied directly with the mobility of the fish (exploratory behaviour) and proximity of reproductive periods. Posit adjustments and very limited motions varied inversely with the duration of feeding and exploratory motion.

Activity was composed of a number of short-term components or 'bouts'. There were seasonal alterations in the length and composition of the activity bouts. These features of the activity of *C. plumbeus* are considered in Appendix III.

B. Photoperiodic Entrainment

(i) Introduction

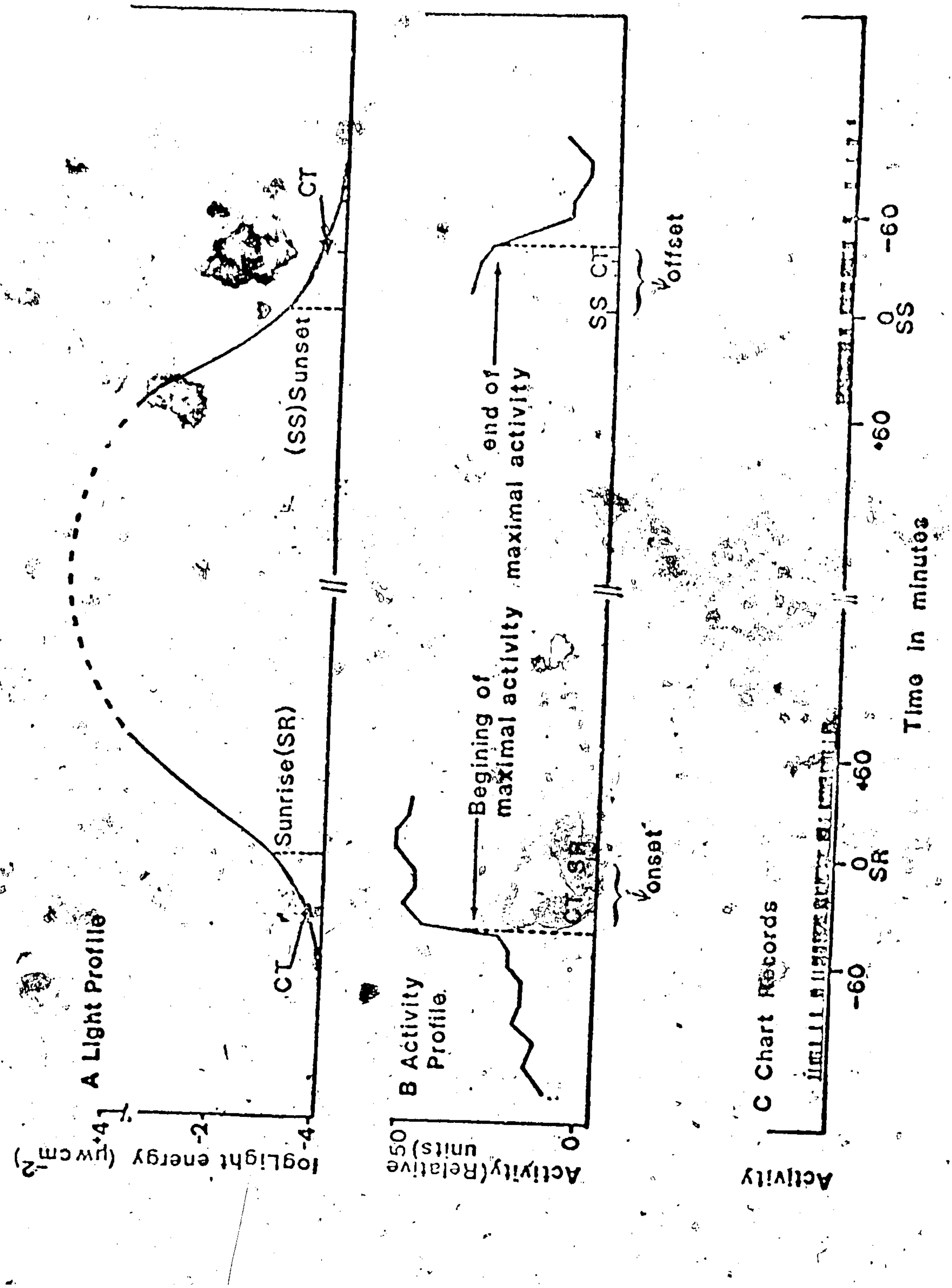
Entrainment of diel and circadian rhythms involves both phase and frequency control (Bünning 1973). The former is expressed in terms of phase angle (Ψ) differences. A phase angle is a relative term expressing, in either degrees or units of time, the distance between a particular point in a biological cycle and some arbitrary reference point in an environmental cycle. Reference points in photoperiods are generally the light-dark transitions or sunrise and sunset. When the biological cycle leads the environmental cycle the phase angle is defined as positive, whereas, when the biological cycle lags behind the environmental cycle, the phase angle is negative. These relationships can be defined for the beginning (Ψ_{onset}) and end (Ψ_{offset}) of activity. Exact definitions and examples of terminology use are illustrated in Figure 18.

Fig. 18. 'Schematic' of Ψ_{onset}, Ψ_{offset}, and the beginning and end of maximal activity of *C. plumbeus*.

A represents the light profile, and indicates the location of Sunrise (SR), Sunset (SS) and Civil Twilight (CT) phase point in the daily photoperiod.

B represents the activity profile, that is, total activity summated over 5 minute intervals. The difference in minutes between the timing of (Beginning of Activity — Sunrise) and (End of Activity — Sunset) [illegible] onset and Ψ_{offset}, respectively.

C represents portion of the chart record from which the activity profile was obtained.


A Light Profile
logLight energy (µw cm-2)
4
-2
-4
CT
Sunrise(SR)
(SS)Sunset
CT
B Activity Profile
Activity(Relative units)
50
0
Begining of maximal activity
end of maximal activity
CT SR
onset
SS CT
offset
C Chart Records
Activity
-60
0 SR
+60
+60
0 SS
-60
Time in minutes

Seasonal changes in the phasing of the beginning and the end of activity were first described from annual changes in bird activity. Aschoff (1960) proposed that daylength, light intensity, and light-dark ratio were the main determinants of seasonal phase relationships, while Wever (1967) suggested that the duration of twilight was the main determining factor. Both Aschoff's and Wever's suggestions have been incorporated into a joint daylight (length)/twilight-duration model of entrainment (Aschoff 1969). Some support for this model has come from analytic studies with artificial twilights (West and Pohl 1973) and analysis of the activity of birds and mammals in the field and in the laboratory under sky lighting (Daan and Aschoff 1975).

The majority of the aforementioned analytic studies with twilight have only considered duration, intensity, and superficially rates of change. Laboratory studies usually have only very rough approximations of twilight duration, often seasonally inappropriate, while field studies have been confounded by the effects of other environmental variables.

Against this background one can proceed with the main experimental topics of this section. Firstly, to examine the seasonal course of the entrainment and phasing of activity of *C. plumbeus* under photoperiods with and without twilights. Secondly, to examine the general roles of twilight in the entrainment of the activity of *C. plumbeus*.

In carrying out these objectives seasonally appropriate duplicated twilights were used, allowing an examination of the roles of different components of dawn and dusk in entrainment.

(ii) Methods

In order to conclusively determine the effects of twilight, activity was observed through several annual cycles of LD and LD+t. The beginning and end of maximal activity (relative to sunrise and sunset) and behavioural composition were the primary locomotory variables observed. Fish used in phase studies were also utilized in free-running period determinations (Chapter V). In the study of circadian activity fish that were entrained under various photoperiods were subject to constant dark for 5-7 days while their locomotory activity was monitored. They were then returned to normal entraining photoperiods (LD or LD+t). This procedure was carried out 3-4 times a year. Subsequent analyses of the activity and phase relationships of fish were not performed until at least 1-2 months after re-entrainment. This procedure was followed in an attempt to ensure return to 'proper' synchrony and eliminate transient behaviours and phase relationships.

A complete annual cycle of activity was not determined for any one fish because of the above manipulatory processes as well as limitations on the number of fish that could be monitored at any one time. Every fish used for phase analysis was examined for at least 3-5 days of each month. Repeatability was tested by carrying out activity determinations over three annual photoperiod cycles (1974, 1975, 1976). Equivalent results were obtained for the three years. A listing of the sex, age, and catalogue number of specific fish used appears in Appendix II. A total of 179 fish were analysed.

(iii) Results

Under seasonally appropriate LD+t and LD photoperiods all of the 87 *C. plumbeus*, whose activity was recorded for periods of 3 days or more, displayed weakly bimodal patterns of diurnal activity (Fig. 19). Observations from outdoor tanks with natural food, substrate and illumination confirmed these laboratory findings. Earlier, preliminary experiments with 5 single lake chub had demonstrated that photoperiod was indeed an effective Zeitgeber of locomotor activity (see Figs. 15 and). Daily fluctuations in water temperature were ineffective as cues (Appendix IV).

The diel locomotor activity patterns of single and groups of *C. plumbeus*, the latter not illustrated, did not have any distinct onsets or offsets of activity; rather, there were changes in the relative amplitude of motion during dawn and dusk and at the L-D and D-L transitions (Fig. 20).

When data records and activity transitions are not wholly clear it is necessary to define specific thresholds or marker events for selecting a relative onset (beginning) and offset (end) of maximal activity. For the purposes of determining phase angles (Ψ), the occurrence of the greatest rate of activity change during a 24-hour period was used to define the onset of activity. This rate was considered to indicate the commencement of maximal activity.

Since the locomotion of *C. plumbeus* was distributed in bout form throughout the day, the maximal level change was not always directly evident. Therefore, it was necessary to use a consistent criterion

Fig. 19. Representative activity of single *C. plumbeus* under photoperiods, A) with twilight (LD+t), and B) without twilight (LD). Both photoperiods have 12 hrs light/12 hrs dark (water temperature, 16°C). The L-D and D-L transitions in the LD records occur at sunrise and sunset, respectively. Light approximated full daylight spectra. C) Enlargements of activity during dawn and dusk. D) Enlargements of activity at L-D and D-L transitions. Examples from 2 days are provided. Activity is in arbitrary units based on 15 min totals for A and B and one (1) min totals for C and D. Time is in hours of Mountain Standard Time (MST). Data are for May 1975.

——— represents activity of fish; ---- represents the course of daily photoperiods in A and B; and the dark-light, light-dark transitions in C and D. (Light energy is expressed as total irradiance (350-750 nm.).

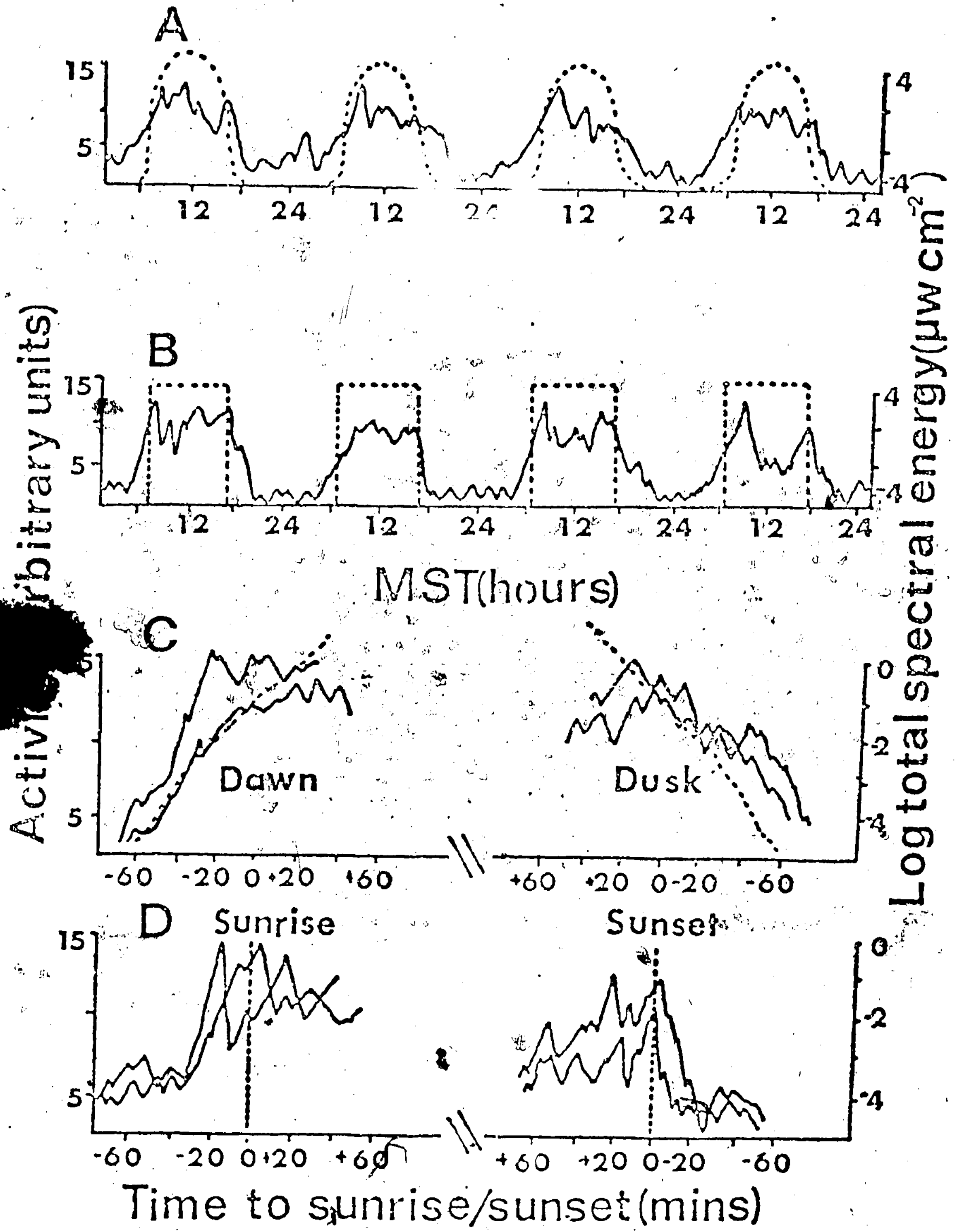

A
B
C
D
15
5
4
-4
12
24
0
-2
MST(hours)
Dawn
Dusk
-60
-20
0
+20
+60
Sunrise
Sunset
Activi[illegible]rbitrary units)
Log total spectral energy(μw cm^{-2})
Time to sunrise/sunset(mins)

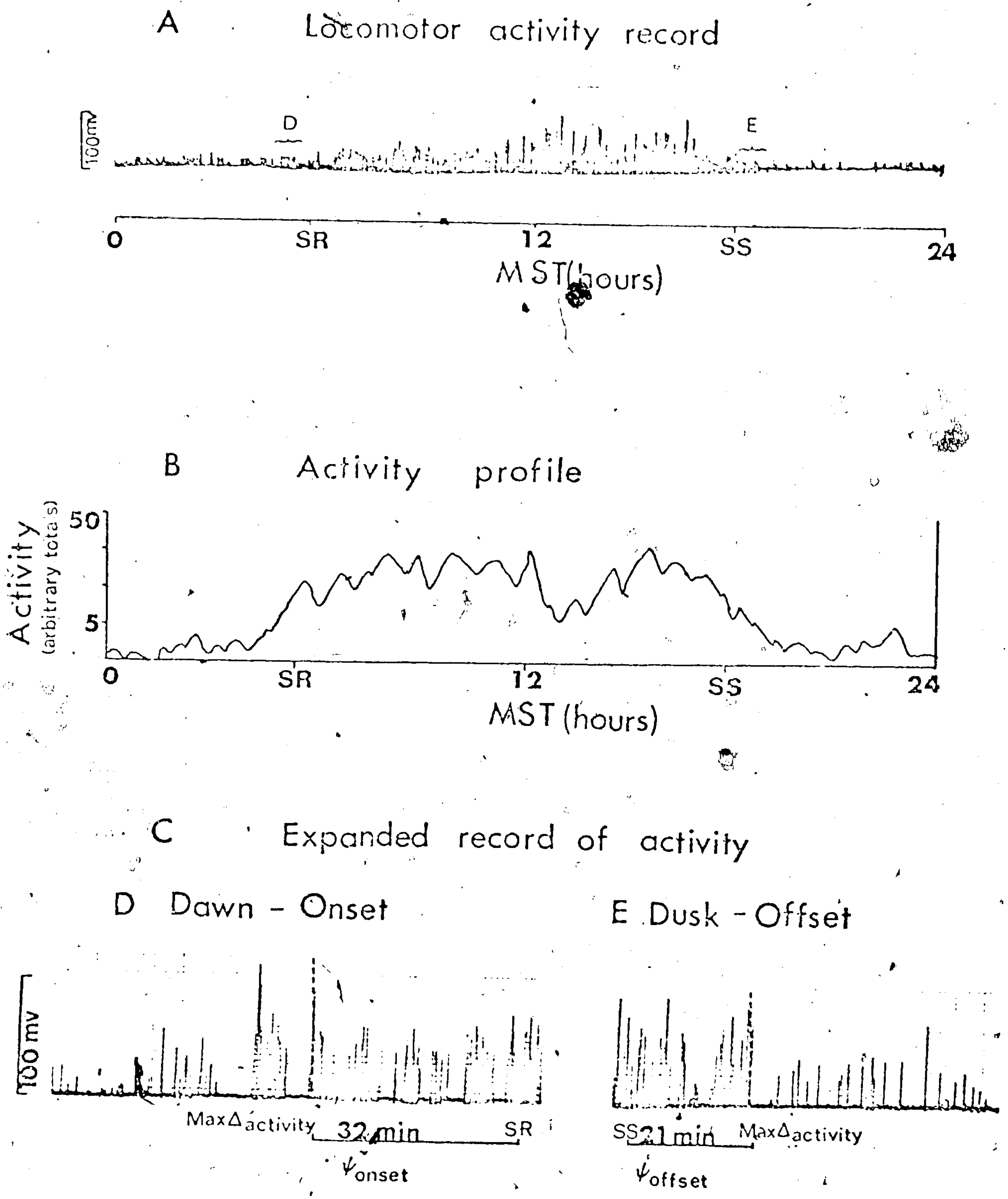
Fig. 20. A Example of a daily chart record of the locomotor activity of a single *C. plumbeus* held under a LD+t photoperiod. The record illustrated is for May 7, 1977.

B Activity profile of the locomotory record shown in A. Activity is plotted as relative peak totals over 10 minute intervals.

C Expanded plots of the sunrise (SR) and sunset (SS) portions of the activity chart records shown in A.

D Record of activity during dawn portion of A, illustrating the maximum rate of increase in activity (Max Δ_{activity}) and Ψ_{onset} relative to sunrise (SR).

E Record of activity during dusk portion of A, illustrating the maximum rate of decrease in activity (Max Δ_{activity}) and Ψ_{offset} relative to sunset (SS).


A Locomotor activity record
100mv
D
E
0
SR
12
SS
24
MST (hours)
B Activity profile
Activity (arbitrary totals)
50
5
0
SR
12
SS
24
MST (hours)
C Expanded record of activity
D Dawn - Onset
E Dusk - Offset
100 mv
MaxΔactivity
32 min
SR
ψonset
SS
21 min
MaxΔactivity
ψoffset

to define this transition. The method adopted for extracting the onset of activity involved scanning or reading the data (activity totals) forward from near the estimated mid-point of the inactive portion until a maximal [illegible] and non-transitory level change (duration greater than three minutes) was found. This point was considered to be the maximal activity transition and is used interchangeably with the term activity onset or beginning of activity. As a check on this determination, activity was read backward in time from mid-activity until a maximum rate of decrease was found. When the two onset points coincided or were close in value, the mean value was considered to indicate the beginning of activity. The reverse procedure of scanning from mid-points was used to determine the offset or end of activity. In computer analysis of the tabulated activity time series onset and offset were taken as occurring at the maximum first derivative of the rate of change of activity.

The onset and offset times were presented relative to sunrise and sunset, i.e., the difference in time from the activity transition is Ψ_{onset} for sunrise and Ψ_{offset} for sunset. Five consecutive Ψ_{onset} and $\Psi_{offsets}$ were used to calculate the mean values and their 95% confidence intervals.

Phase relations of the mid-point of activity were not provided, primarily because of technical problems in obtaining reliable estimates from the 'bout-like' distribution of activity (Appendix III). Daan (1976), Kenagy (1976), and Pohl (1976) outline difficulties and assumptions inherent in the determination of mid-point phase values from variable data records.

Examples of locomotor records from which Ψ values were determined, are shown in Figure 20. Enlargements of the records from the transition times indicate the positions at which greatest rates of change were determined by visual inspection. In these representative portions of data Ψ values were also determined by computer analyses. The values found by computer and visual analyses are listed in Table 3. No significant differences were evident between the values obtained by the two methods.

Computer determinations of the timing of the maximum first derivative of the slope were obtained only when the data were compiled and processes for additional analyses (Appendix III). In order to maintain consistency of interpretation and the same level of interpretation, all Ψ relationships listed and subject to further analyses are based on visually determined values. However, regular checks on the visual interpretations were made with the computer technique.

The beginning and end of the activity of *C. plumbeus* followed the seasonal course of twilight and daylength, respectively (Tables 4 and 5; Figures 21, 22, 23). Similar patterns were evident for all years examined (1974, 1975, 1976).

Onset and offset of maximal activity occurred during the twilight portions of the LD+t photoperiods (see Figs. 21, 22, 23). Under LD photoperiods the onset of activity was shown as an apparent anticipatory change in the amplitude of motion prior to the actual dark to light transformation (see Fig. 20B).

Differences between the beginning of activity and sunrise (Ψ_{onset}) as well as the end of activity and sunset (Ψ_{offset}) are

Table 3. Comparisons of Ψ_{onset} and Ψ_{offset} values obtained by 'visual' interpretations and computer analyses of the locomotory activity records of *C. plumbeus*.

Time of Year	Ψ_{onset} (minutes)		Ψ_{offset} (minutes)	
Month	Visual	Computer	Visual	Computer
Jan	14	13	12	11
Mar	41	42	24	23
May	36	37	40	42
Jul	34	34	47	46
Sep	48	46	29	30
Nov	23	25	10	11

[1] All results were obtained from LD+t activity records.

[2] Visual determinations were based on the times of the greatest rate of change in activity. Computer determinations were based on the time of occurrence of the maximum first derivative of the rate of change of activity. All values are based on the mean values of five consecutive onset and offset readings. Three fish were compared each month; however, data are presented only for 1 fish.

[3] No significant differences were evident between the values obtained by visual and computer determinations (Mann-Whitney U test, $p = 0.05$).

Table 4. Beginning of activity relative to sunrise (Ψ_{onset}) and end of activity relative to sunset (Ψ_{offset}) for single *Couesius plumbeus* under LD+t photoperiods.

Fish #	Sex	Age Class (years)	Ψ_{onset}[1,2] ([illegible]ne) and Ψ_{offset}[1,2] (bottom line) (minutes)											
			Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
C6	M	3		37±12 13±7	47±16 17±12	45±12 21±13	41±10 37±16	22±6 39±16	37±16 46±21		44±18 26±11	30±11 28±11	22±5 9±5	15±5 11±5
C7	F	2	16±5 8±5		37±13 16±11	34±9 20±11	30±8 28±11	15±7 26±12	26±11 23±8	35±7 34±16		22±6 29±12	17±5 12±7	12±5 6±5
C12	M	2	24±8 9±5	40±13 17±9		49±17 22±7	41±13 35±13	18±7 37±13		37±12 51±17	36±12 37±16		22±7 18±6	20±9 7±5
C15	F	3		25±10 12±5	37±12 25±12		32±10 41±17	17±6 47±12	30±12 49±16	37±13 57±22		21±10 28±13		16±7 12±5
C18	-	2	22±7 7±7	35±9 13±11	52±12 24±10	54±17 28±12		31±11 42±17	43±15 49±17	55±17 41±18	52±18 36±13	32±12 27±12	26±8 13±7	
C22	F	3	18±4 9±6		42±10 21±8	37±9 22±13	33±12 37±16		35±10 45±13	39±11 54±16	41±15 31±12	36±9 28±13	28±6 17±8	17±8 6±5
C27	M	2	26±6 11±5	39±12 16±13		42±12 21±8	36±13 41±24	24±10 49±21		42±13 44±13	45±11 36±7	29±9 32±12	17±5 18±9	13±5 7±7
C29	M	2		34±17 18±16	45±13 22±11	41±11 29±17	35±8 47±21	27±9 53±24	36±12 54±21		40±13 42±21	21±7 27±12	14±5 11±7	10±5 7±5
C32	F	2	16±5 13±7	27±8 16±13	42±12 22±7	44±16 29±12		14±8 45±17	27±11 38±10	36±13 41±16		20±6 31±17	18±13 17±6	16±7 19±5
C38	M	2	20±6 17±6	25±7 18±12		36±13 16±8	30±17 31±16	22±5 39±18	35±11 49±17	44±12 47±17	46±13 32±16		27±7 12±13	13±5 7±5
C40	F	2	22±7 12±6	31±9 17±11	42±13 28±12		32±7 49±21	24±8 54±22	31±9 58±13	51±14 50±17	55±11 52±18	36±9 37±12		21±7 11±5
C41	M	-	18±8 7±5	37±11 12±7	52±17 22±10	49±16 39±16	41±13 54±22		32±8 62±21	46±13 57±20	42±7 41±12	29±8 27±9	21±9 8±7	
Mean Ψ_{onset}			20.2 ±7	33.1 ±10	44.2 ±15	43.3 ±13	35.1 ±11	21.6 ±8	33.2 ±13	42.3 ±12	40.0 ±13	27.6 ±9	21.2 ±8	15.3 ±6
Mean Ψ_{offset}			11.1 ±7	15 ±11	20.7 ±10	5.4 ±13	40.2 ±17	43.4 ±16	47.3 ±16	48.6 ±17	34.5 ±13	29.4 ±12	12.5 ±8	8.3 ±5

[1] Plus and minus values (±) represent 95% confidence interval of 5 consecutive values.

[2] Blank spaces represent times at which free-running period (Tau) determinations were carried out. Values are provided in Table 6.

Table 5. Beginning of activity relative to light onset (sunrise) (Ψ_{onset}) for single *Couesius plumbeus* under LD photoperiods.

Fish #	Sex	Age Class (years)	$\Psi_{onset}^{1,2}$ (minutes) Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
C5	M	2		31±16	21±12	16±8		13±8	22±12	29±13	31±12	17±5	16±8	25±12
C8	F	3	27±13		22±12	29±9	31±16		27±12	14±7	16±8	17±9	18±13	19±13
C9	M	1	35±12	31±12		16±8	17±4	18±9	29±12		31±14	24±11	17±9	18±12
C16	M	1	12±7	17±9	23±5		29±12	43±19	22±16	23±7	17±8		19±7	22±8
C23	-	1	16±9	18±11	19±7	23±12		29±16	27±13	28±12		19±6	17±8	25±11
C25	F	2	16±12	17±8	19±12	22±8	24±9		17±12	19±13	16±15	23±7		24±13
C28	M	3	23±16	21±9	28±16	22±13	17±16	18±17		22±13	28±12	6±12	9±8	
C30	M	3		13±12	22±16	25±12	16±13	14±12	19±13		16±12	14±9	18±17	9±7
C33	F	2	16±9		34±16	37±18	14±16	22±13	28±16	24±13		27±16	28±9	24±13
C34	M	1	14±16	15±13	22±18		27±13	26±15	28±13	27±13	22±16		21±12	16±13
C35	-	1	16±9	19±13	28±11	17±13	14±12		17±13	16±8	18±12	14±11		16±12
C36	M	2	17±8	19±16		19±16	17±12	14±13	23±16	29±18	22±16	25±13	21±16	
Mean Ψ_{onset}			17.6 ±11	20.4 ±12	23.8 ±14	22.6 ±13	20.6 ±14	19.7 ±11	25.9 ±15	23.1 ±12	21.7 ±13	18.6 ±10	18.4 ±10	20.0 ±12

[1] Plus and minus values (±) represent 95% confidence intervals of 5 consecutive values.

[2] Blank spaces represent times at which free-running period (Tau) determinations were carried out. Values are provided in Table 7.

Note: Fish were entrained under laboratory photoperiods without twilights (LD). Values are for 1975.

Fig. 21. Annual and monthly changes in the locomotory activity profiles of *C. plumbeus*. Examples of pre- and post-sunrise and sunset activity are shown in arbitrary units of activity.

··· represents activity under LD photoperiods.

—— represents activity under LD+t photoperiods.

--- represents annual time course of sunrise and sunset.

Mean records of 5 fish from 1975 were used.

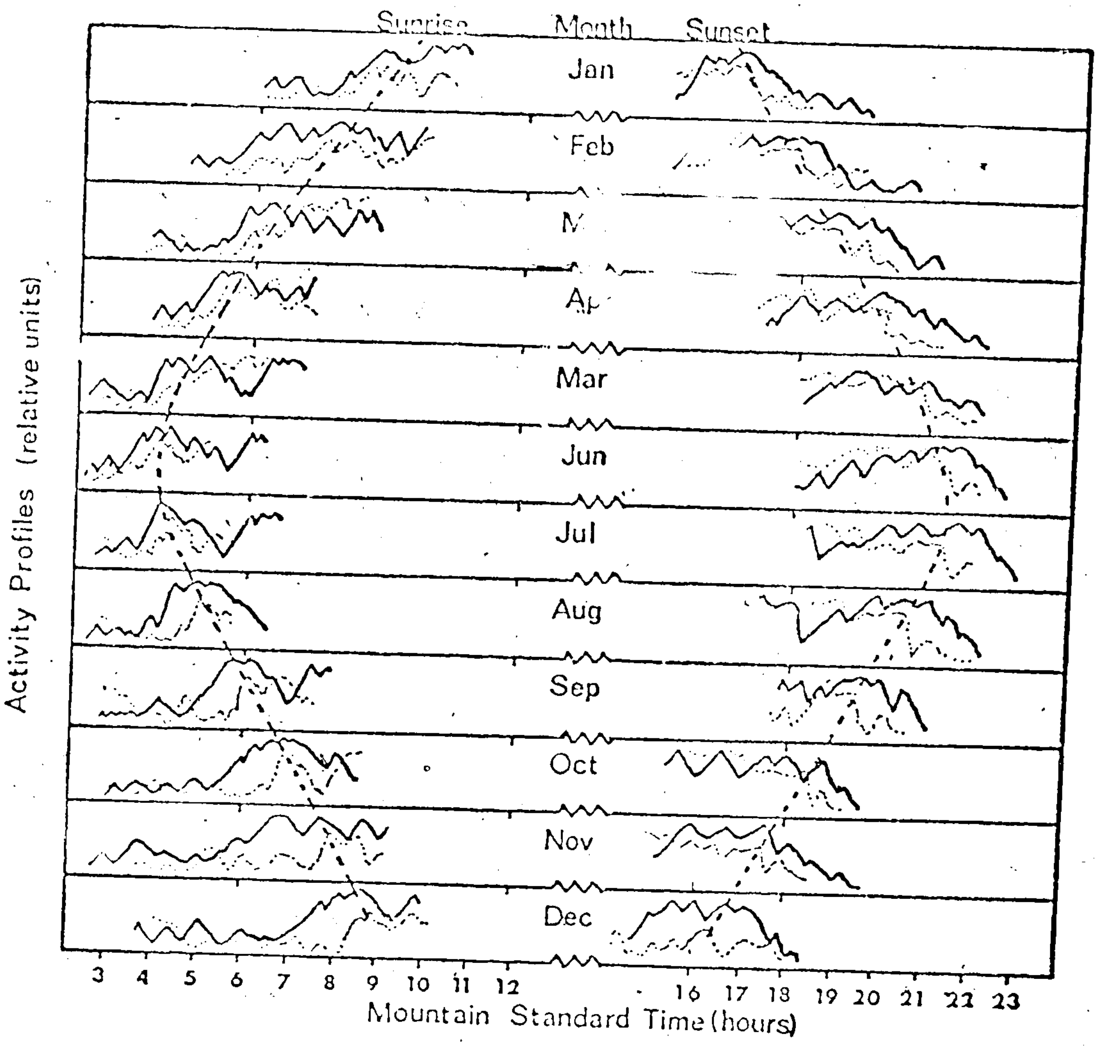

Sunrise
Month
Sunset
Jan
Feb
M
Ap
Mar
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Activity Profiles (relative units)
3
4
5
6
7
8
9
10
11
12
16
17
18
19
20
21
22
23
Mountain Standard Time (hours)

Fig. 22. A Beginning and end of maximal activity for each *C. plumbeus* examined under photoperiods with twilights (LD+t) (see Table 4). The mean monthly values (·) of each individual fish are used. MST represents Mountain Standard Time.

B Beginning and end of maximal activity for each *C. plumbeus* examined under photoperiods without twilights (LD) (see Table 5). The mean monthly values (·) of each individual fish are used. MST represents Mountain Standard Time.

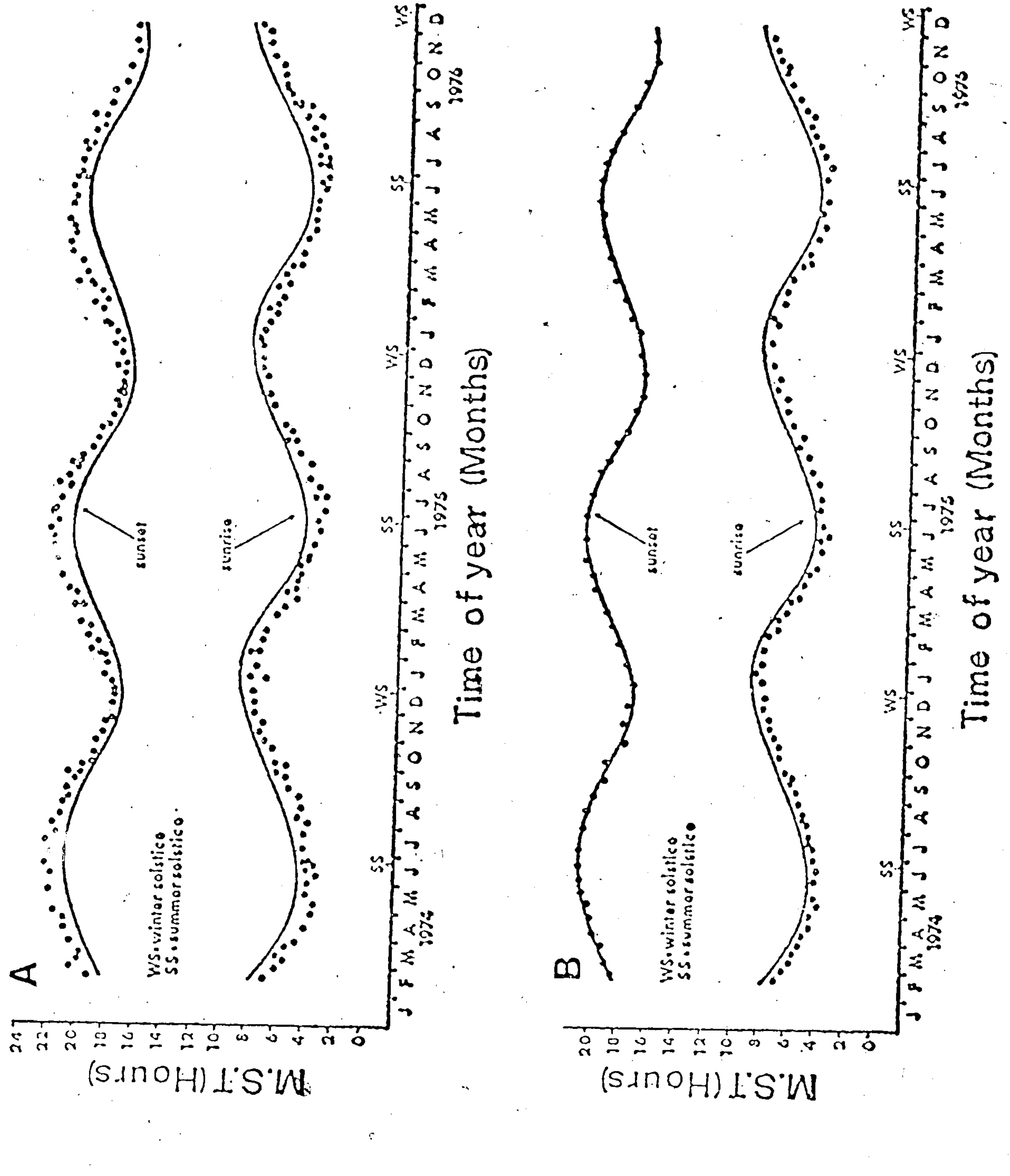
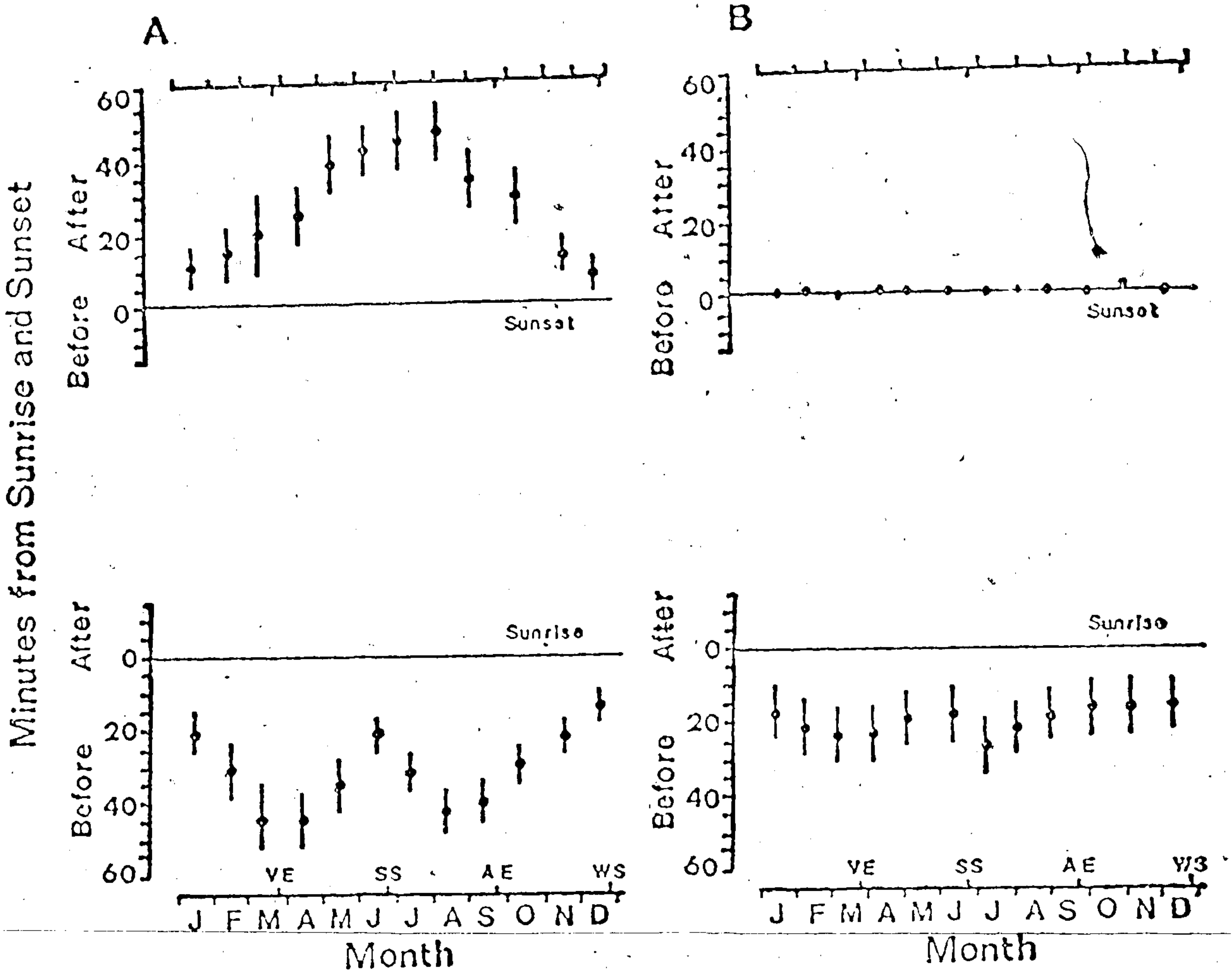

A
B
M.S.T (Hours)
24
22
20
18
16
14
12
10
8
6
4
2
0
WS = winter solstice
SS = summer solstice
sunset
sunrise
SS
WS
J F M A M J J A S O N D
1974
1975
1976
Time of year (Months)

Fig. 25. A Differences between the beginning of activity and sunrise (below) and end of activity and sunset (above) for single *Couesius plumbeus* from photoperiods with twilights (LD+t). Mean monthly average readings for individual fish are used (n=12).

B Differences between the beginning of activity and sunrise (below) and end of activity and sunset (above) for single *Couesius plumbeus* from photoperiods without twilights (LD). Mean monthly average readings for individual fish are used (n=12).

VE = vernal equinox; SS = summer solstice; AE = autumnal equinox; WS = winter solstice.

All values are for 1975.


A
B
Minutes from Sunrise and Sunset
60
40
20
0
After
Before
Sunset
Sunrise
VE
SS
AE
WS
J F M A M J J A S O N D
Month

listed in Tables 4 and 5. No Ψ_{offset} values are presented for LD conditions as the activity change took place at the actual light to dark transition. The monthly mean phase value and 95% confidence intervals of each fish are based on 5 consecutive onset or offset values. Monthly inter- and intra-fish differences in the timing of activity were not significant (Mann-Whitney U test $p > 0.20$), thus permitting a pooling of samples. No significant differences were evident between different sexes and ages of fish. Mean activity onsets and offsets were used in the graphical representations (see Figs. 22 and 23). Repeatability was tested by determining all phase relationships over 3 years.

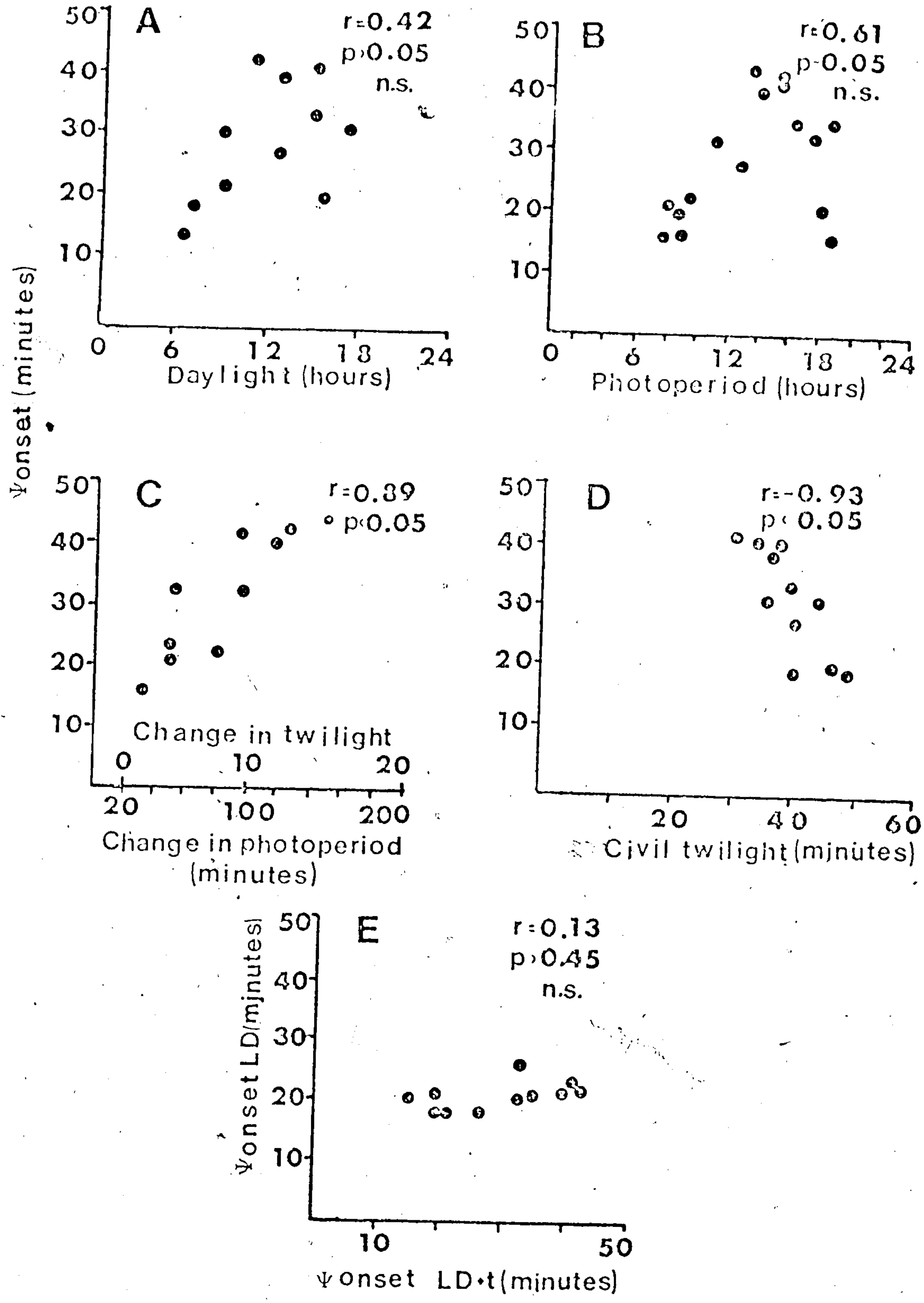
The beginning of activity of fish held under LD photoperiods was considerably more variable than that of individuals from LD+t. There was generally more variation in offset than onset of activity under LD+t.

Activity began earliest relative to sunrise (Ψ_{onset} minimal) in early spring and the vernal equinox and latest at the summer solstice (Ψ_{onset} maximal). The winter solstice and autumn equinox represent the other timing minima and maxima, respectively (see Fig. 23).

Timing of onset (Ψ_{onset}) was examined in relation to durations of photoperiod, daylength, and twilight (Fig. 24). The relationships are presented only for mean values, but they are equivalent to the values obtained by utilizing phase values of individual fish.

The bimodal seasonal course of change in Ψ_{onset} resembles the change in length of twilight in that both show two cycles per year (see Fig. 23A). A highly significant relationship was evident between ig. 24. Relationships between the timing of the beginning of activity (Ψ_{onset}) and various photoperiodic parameters for single *Couesius plumbeus* entrained under photoperiods with twilight (LD+t).

	Parameter	Relationships	
A)	daylight or daylength	$p > 0.05$	n.s.
B)	photoperiod duration	$p > 0.05$	n.s.
C)	rate of twilight change	$p < 0.05$	sig. ($r = 0.89$)
D)	civil twilight duration	$p < 0.05$	sig. ($r = 0.93$)
E)	nonsignificant relation between onset and offset Ψ values	$p > 0.45$	n.s.


A
r=0.42
p>0.05
n.s.
B
r=0.61
p>0.05
n.s.
Ψonset (minutes)
0
6
12
18
24
10
20
30
40
50
Daylight (hours)
Photoperiod (hours)
C
r=0.89
p<0.05
Change in twilight
0
10
20
20
100
200
Change in photoperiod (minutes)
D
r=-0.93
p<0.05
20
40
60
Civil twilight (minutes)
E
r=0.13
p>0.45
n.s.
Ψonset LD (minutes)
10
50
Ψonset LD+t (minutes)

civil twilight length and the timing of the beginning of maximal activity ($r = -0.932$; $p < 0.05$) (Fig. 24D). No significant relationship was evident between the timing of activity onset and daylength (Fig. 24A), while a marginal relationship was evident between photoperiod duration and activity onset (Fig. 24B). Kendall's partial correlation analysis (Sokal and Rohlf 1980) revealed that the latter relationship was spuriously generated by the presence of twilight components in photoperiods. A significant relationship was also evident ($r = 0.89$; $p < 0.05$) between the rate of change in twilight duration and Ψ_{onset}.

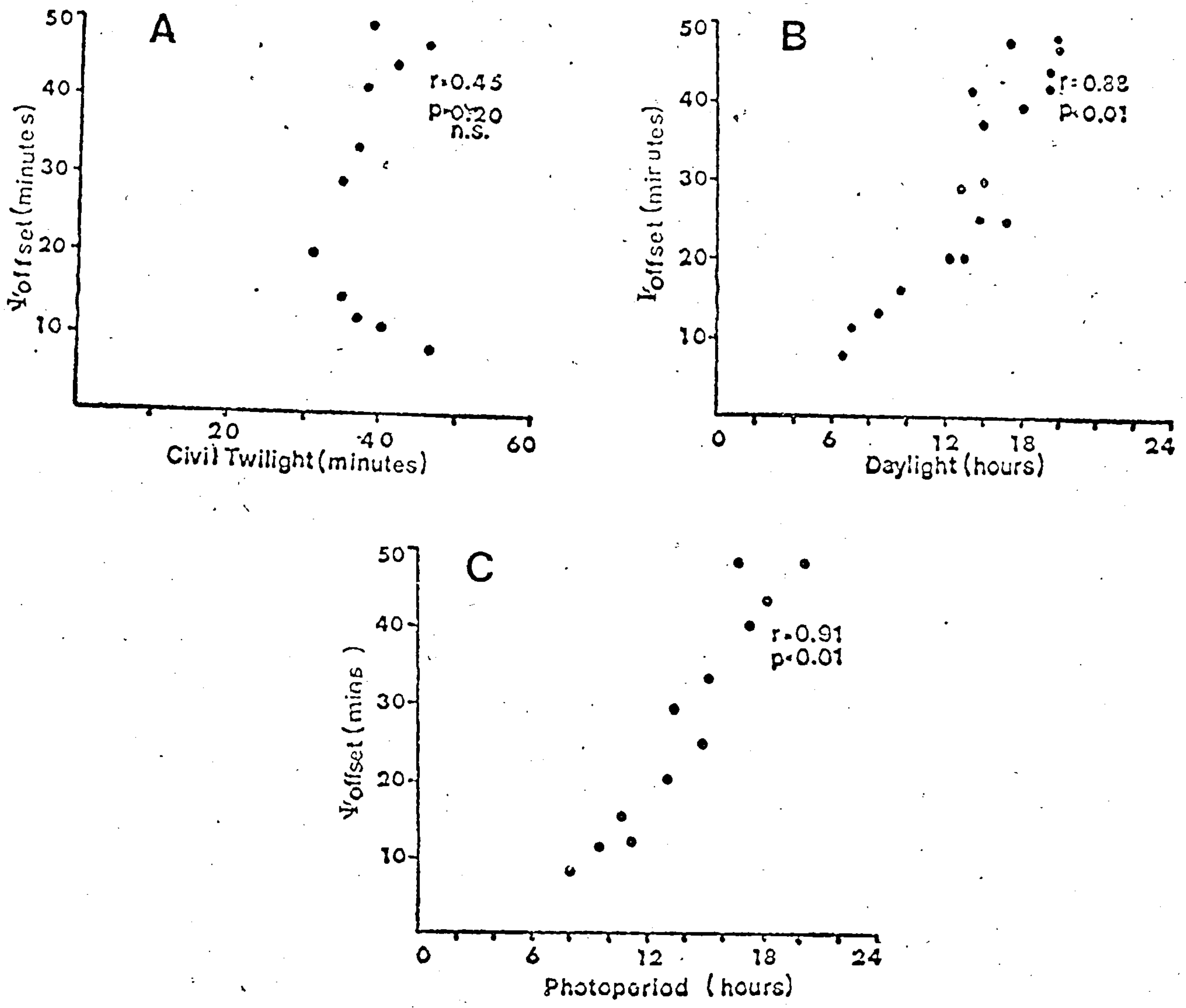
Seasonal variations in the timing of the end of activity are shown in Figure 25. Activity termination always occurred during dusk after sunset. Activity ended earliest relative to sunset (Ψ_{offset} minimal) at the winter solstice and latest relative to sunset (Ψ_{offset} maximal) at the summer solstice.

The timing of the end of activity was examined in relation to the duration of daylight, photoperiod, and twilight (Fig. 25). A highly significant relationship ($r = 0.896$; $p < 0.01$) was found between daylight and Ψ_{offset} (Fig. 25B).

A significant relationship (regression) was present between Ψ_{offset} and photoperiod length (Fig. 26C; $r = 0.91$; $p < 0.05$; $y = 6.25 + 3.7x$). Covariance, slope, and elevation of this regression were not different between increasing and decreasing photoperiod lengths. This indicated that the direction of photoperiod change (increasing or decreasing daylength) did not significantly affect the timing of the end of activity. No significant relationships were

Fig. 25. Relationships between the timing of the end of activity (Ψ_{offset}) and various photoperiodic parameters for single *Couesius plumbeus* entrained under photoperiods with twilight (LD+t).

	Parameter	Relationships	
A)	civil twilight duration	$p < 0.20$	n.s.
B)	daylight	$p < 0.01$	$r = 0.88$
C)	photoperiod duration	$p > 0.01$	$r = 0.91$


A
Ψoffset (minutes)
50
40
30
20
10
20
40
60
Civil Twilight (minutes)
r=0.45
p=0.20
n.s.
B
Ψoffset (minutes)
50
40
30
20
10
0
6
12
18
24
Daylight (hours)
r=0.88
p<0.01
C
Ψoffset (mins)
50
40
30
20
10
0
6
12
18
24
Photoperiod (hours)
r=0.91
p<0.01

evident between the timing of the beginning and the end of activity.

Seasonal variations in the timing of activity onset and offset are shown in Figure 21. Activity onset did not show any consistent seasonal pattern or relationship to photoperiod parameters (Fig. 26). The beginning of activity occurred as a consistent anticipatory component in all records, thus generating an apparent relationship to daylength (Fig. 26B).

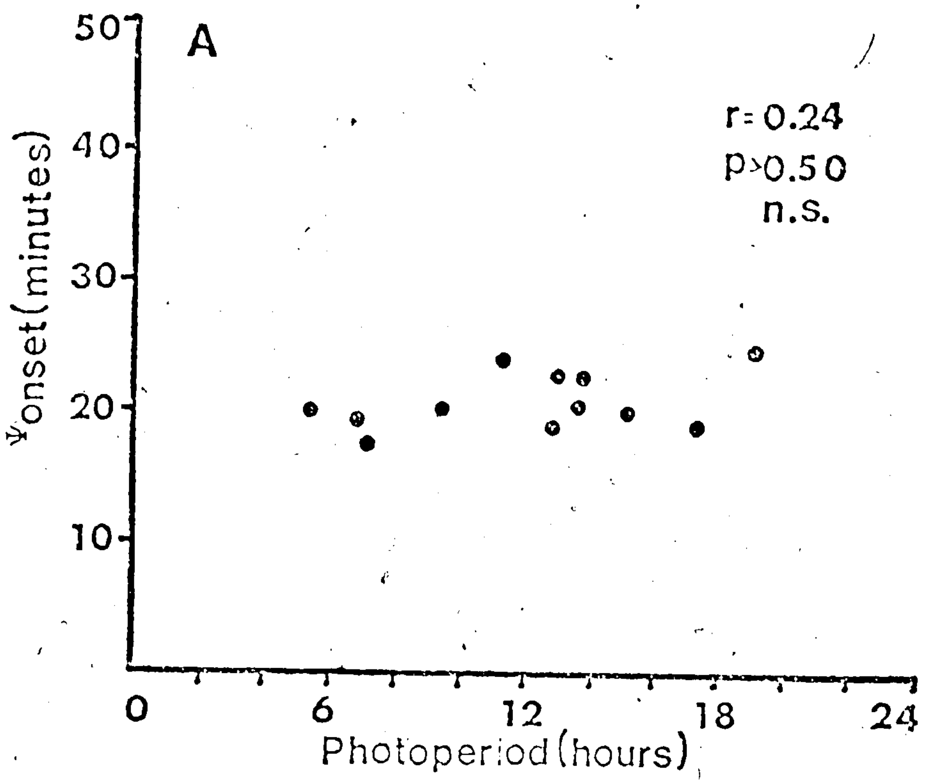
The beginning of maximal activity of *C. plumbeus* did not show any significant relationship to total light energy (Fig. 27). A 10^3-fold difference existed between maximum and minimum light energy found at the beginning of activity.

The end of activity (Ψ_{offset}) from LD+t photoperiods was significantly correlated ($r = 0.86$; $p < 0.01$) with total light energy. There was a 10^2-10^3-fold difference between the extreme maximal and minimal light energies found at the apogee and perigee of Ψ.

The composition and characteristics of light present at all portions of simulated and natural twilights were measured. Seasonal and daily shifts in the timing of various spectral intensities, photon numbers, spectral ratios, rates of spectral intensity and total intensity change were present (Tables 6 and 7). Onset of maximal activity consistently occurred at the following concurrent rates of spectral energy change: 0.4, 0.7, 0.8, and 0.5 (μw cm^{-2} nm^{-1})10^{-2} for 425, 525, 575, 650, and 750 nm spectral regions, respectively. Spectral energy ratios were: 1.55, 1.35, and 1.45 for 725/650, 550/450, and 400/375 nm bands, respectively (Tables 6 and 7). These

Fig. 26. A Nonsignificant relationships between the timing of activity onset (Ψ_{onset}) and photoperiod for *C. plumbeus* from photoperiods without twilight (LD) ($p > 0.05$).

B Nonsignificant relationship between the timing of activity onsets (Ψ_{onset}) for *C. plumbeus* from LD and LD+t photoperiods ($p > 0.05$).


A
r=0.24
p>0.50
n.s.
50
40
30
20
10
Ψonset (minutes)
0
6
12
18
24
Photoperiod (hours)

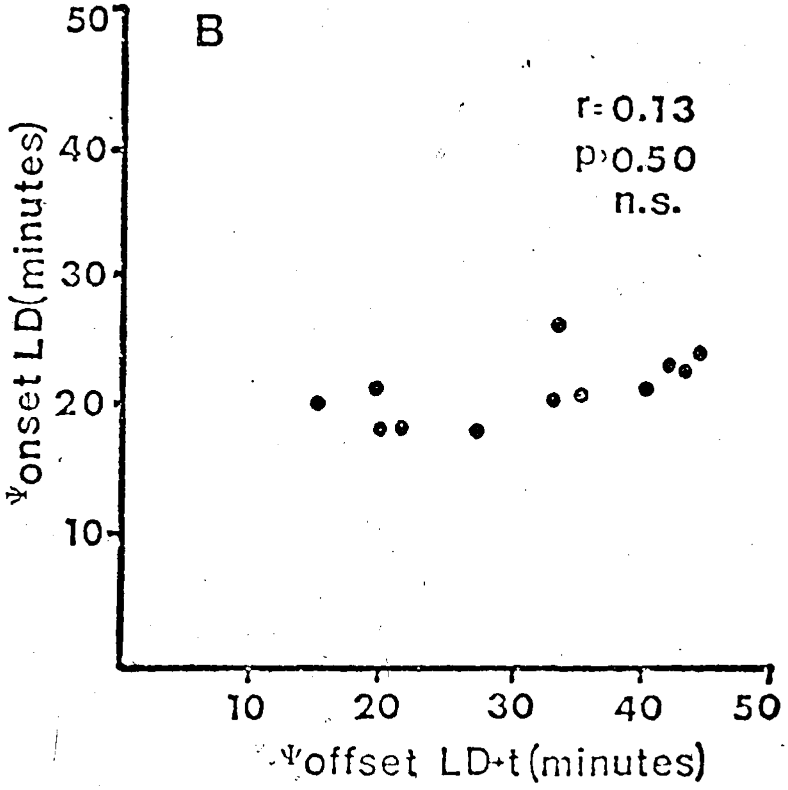

B
r=0.13
p>0.50
n.s.
50
40
30
20
10
Ψonset LD (minutes)
10
20
30
40
50
Ψoffset LD+t (minutes)

Fig. 27. A Significant correlation ($r = 0.86$; $p < 0.01$) between activity offset (Ψ_{offset}) of *C. plumbeus* and total light energy at that time for LD+t.

B Nonsignificant relationship ($p > 0.20$) between activity onset (Ψ_{onset}) of *C. plumbeus* and total light energy at that time for LD+t.

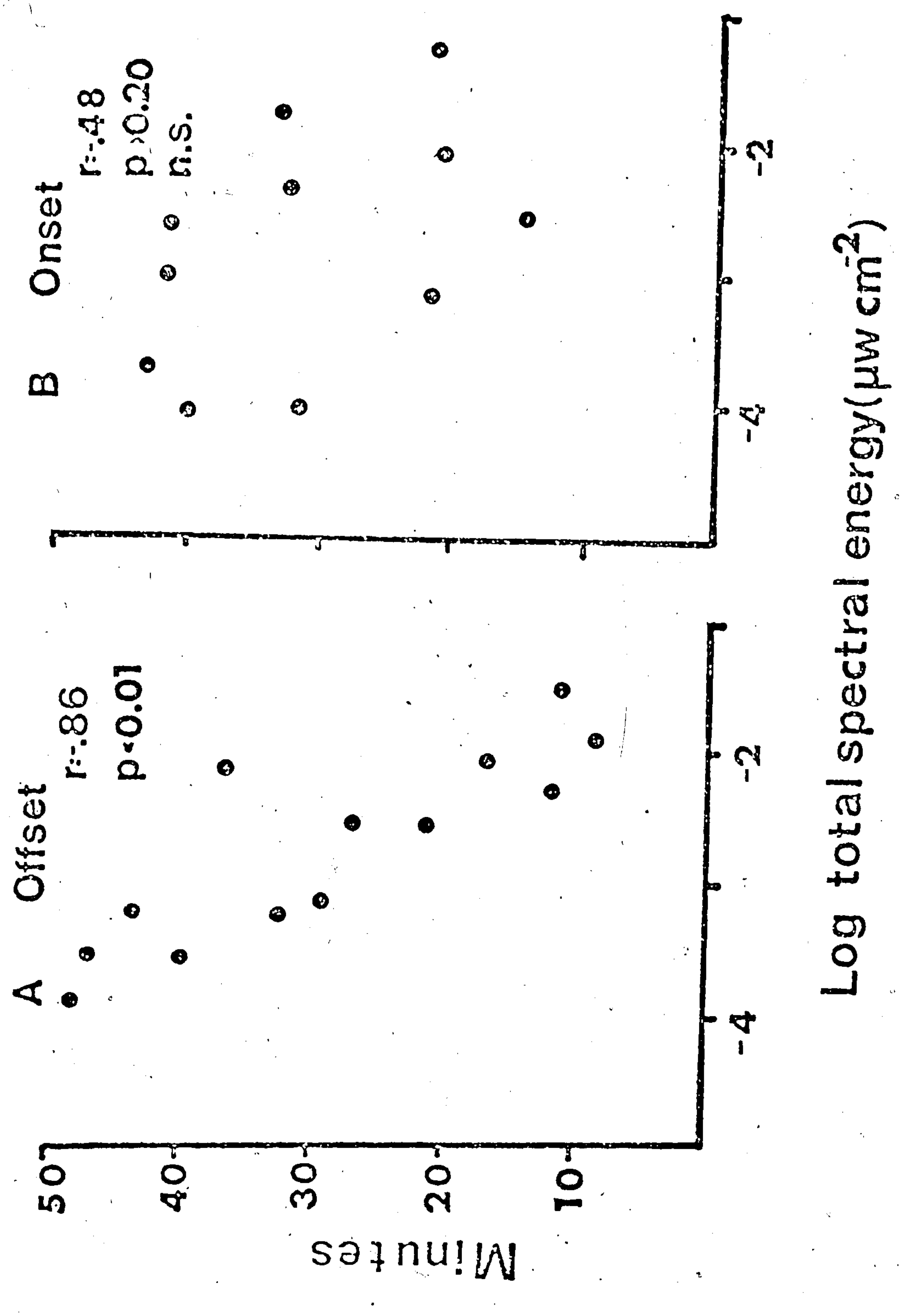

A Offset
r=-.86
p<0.01
B Onset
r=-.48
p>0.20
n.s.
50
40
30
20
10
Minutes
-4
-2
Log total spectral energy (μw cm-2)

Table 6. Rate of change of spectral energy at the beginning of maximum locomotory activity of *Couesius plumbeus*. Energy values are in μw cm^{-2} nm^{-1} min^{-1} sec^{-1}.

Wavelength (nanometers)	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
350	.057	.089	.076	.089	.056	.021	.043	.047	.063	.041	.059	.061
375	.062	.053	-	.041	-	.071	-	.061	-	.023	.037	.042
400	.038	.047	.073	.075	.043	.047	.048	.076	.061	.052	.053	.043
425	.034	.035	.037	.041	.039	.041	.037	.039	.036	.039	.035	.037
450	.048	.041	.036	.037	.038	.045	.047	.049	.046	.042	.043	.041
475	.059	.026	.029	.049	.058	.056	.071	.052	.041	.079	-	.057
500	.071	.046	.039	.052	.073	.076	.089	.071	.072	.073	.071	.093
525	.051	.059	.061	.071	.081	.071	.073	.074	.076	.075	.073	.072
550	.089	.073	.056	.065	.071	.083	.095	.076	.098	.054	.056	.053
575	.072	.075	.076	.080	.081	.085	.079	.087	.086	.065	.067	.089
600	.083	.071	.087	.076	.087	.037	.038	.037	.036	.037	.023	.027
625	.097	.015	.017	.023	.029	.027	.039	.041	.044	.045	.056	.071
650	.065	.067	.067	.051	.057	.063	.067	.068	.061	.057	.065	.063
675	.067	.068	.065	.063	.071	.031	.091	.034	.071	.072	.061	-
700	.01	.081	.072	.092	.13	.089	.051	.072	.082	.091	.072	.082
725	.02	.070	.095	.035	.076	.043	.049	.048	.047	.046	.049	.058
750	-	-	-	.034	-	-	.072	-	-	-	.071	.041

[1] Ψ_{onset} values listed in Table 3 were used as timing measure.

[2] Rate of intensity change is the same for each month.

Table 7. Examples of spectra intensity ratios at the beginning of maximum locomotory activity of *Couesius plumbeus* under LD+t.

Wavelength (nanometers/ nanometers)	Spectral Intensity Ratios[1]											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
750/650 (FR/R)	1.58	1.52	1.47	1.53	1.58	1.56	1.52	1.57	1.53	1.52	1.47	1.45
500/450	1.09	1.53	1.1	1.21	1.21	1.25	1.27	1.29	1.27	1.16	1.15	1.27
400/375	1.34	1.47	1.37	1.56	1.43	1.47	1.42	1.43	1.37	1.39	1.42	1.43

[1] Ψ_{onset} values listed in Table 3 were used as timing measures.

particular rates of energy change and spectral energy ratios were consistently present at the beginning of maximal activity throughout the course of the year. No significant relationships were found between the timing of the end of activity and any particular single spectral component.

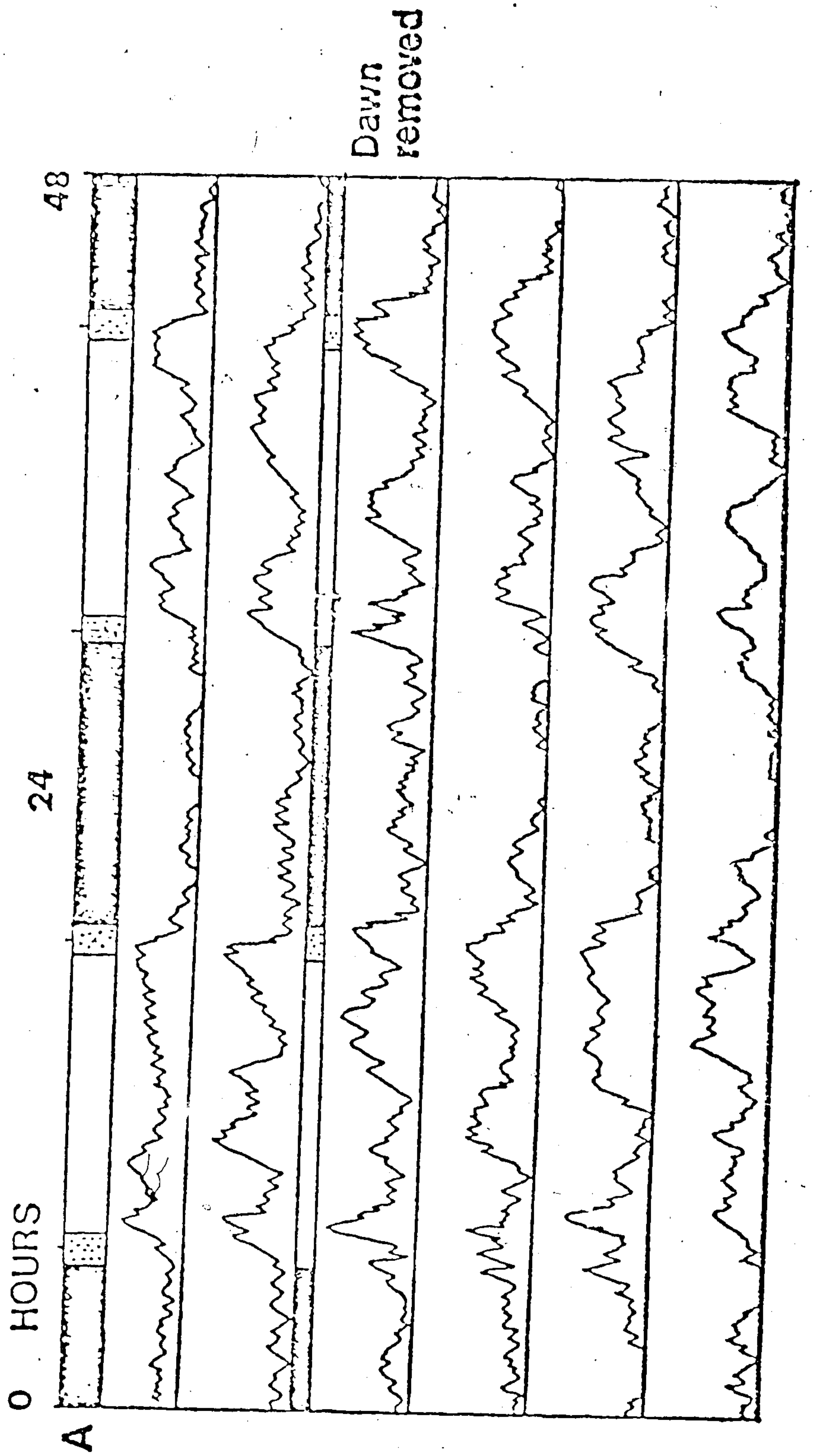
The following analytical procedures were performed with single (n=10) *C. plumbeus* that were exposed to seasonally appropriate LD+t photoperiods. At arbitrarily selected times from the annual photoperiod either dawn or dusk was removed and replaced by a LD transition set either to sunrise/sunset or civil twilight. Water temperatures normally occurring in the field were maintained (Appendix I).

When dusk was abolished the end of activity became a very abrupt decrease in the amplitude of activity that was coincident with the L-D transition and similar to the ones seen under LD entrainment (Fig. 28A).

Replacement of dawn by a dark-light (D-L) transition led to an immediate 'light-shock' reaction (Fig. 28B). Similar results were obtained from all fish (10) that were examined at various times of the year. After 7-10 'transient' cycles the shock reaction disappeared and was replaced by an anticipatory change in maximal activity, similar to that found under LD entrainment (see, for example, Fig. 21).

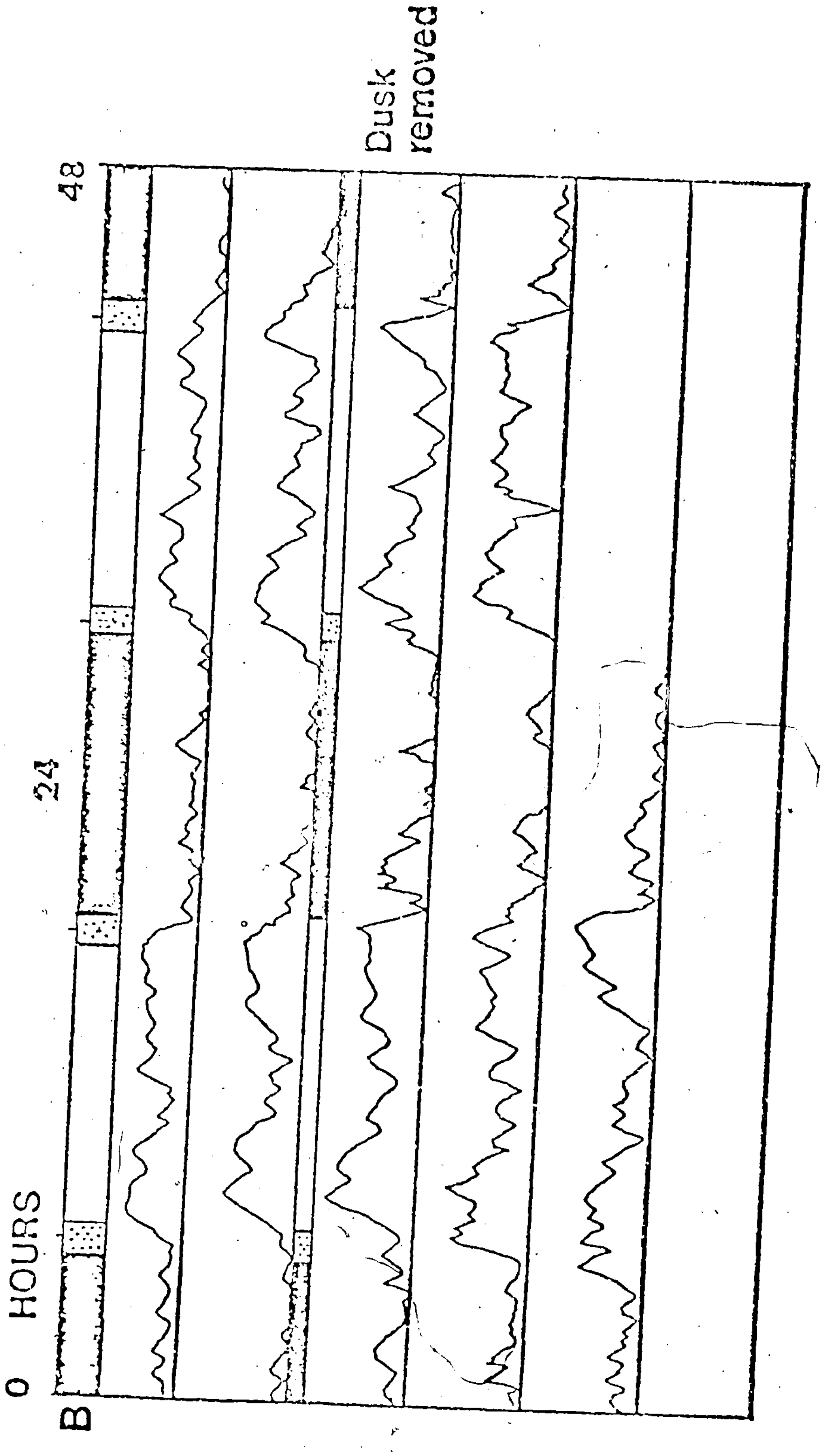
ig. 28. A Locomotory activity of single *Couesius plumbeus* after removal of dawn. Fish were previously kept under 12:12 LD+t photoperiods. The dawn portion was removed and replaced by a Dark-Light (D-L) transition at civil twilight.

(Cont'd)



(Cont'd)

Fig. 28. B Locomotory activity of single *Couesius plumbeus* after removal of dusk. Fish were previously kept under 12:12 LD+t photoperiods. The dusk portion was removed and replaced by a Light-Dark (L-D) transition at civil twilight.


B
0
HOURS
24
48
Dusk
removed

C. The Roles of Various Components of Twilight

(i) Methods

The efficiency as Zeitgebers of different rates of spectral energy change occurring during twilights was analytically examined. A brief description of the methods employed is necessary before the results can be considered.

Determinations of spectral energy changes and/or photon thresholds for inducing changes in the locomotory activity of *C. plumbeus* were carried out in a plexiglass tank (30x50x30 cm) that was placed in a grounded aluminum chamber (Fig. 29). Openings that could be sealed to eliminate stray light were present in the chamber for insertion of light probe, air lines, and terminals from the activity detectors in the tank. A diffusing lens (Frensel Plastic, Toronto) and translucent diffusing screen (Lucite, Toronto) were placed above the tank, 5-10 cm below the top of the chamber. These filters were used to obtain a uniform distribution of light within the tank (Fig. 30). Irradiance characteristics were regulated by spectral and neutral density filters (Optics Technology, California) placed on top of the chamber. Illumination was provided by high intensity incandescent lights (General Electric X-100 175 Watts). The intensity of light was regulated by an adjustable motor driven rheostat (Hammond, Chicago). Heat filters (5% copper sulfate solution) were placed in front of the lights. Water temperature was further controlled by a flow of air above the tanks.

Light characteristics were standardized and calibrate by determining the transmission curves of the filters (Fig. 30) and measuring

Fig. 29. Apparatus used to determine spectral effects.

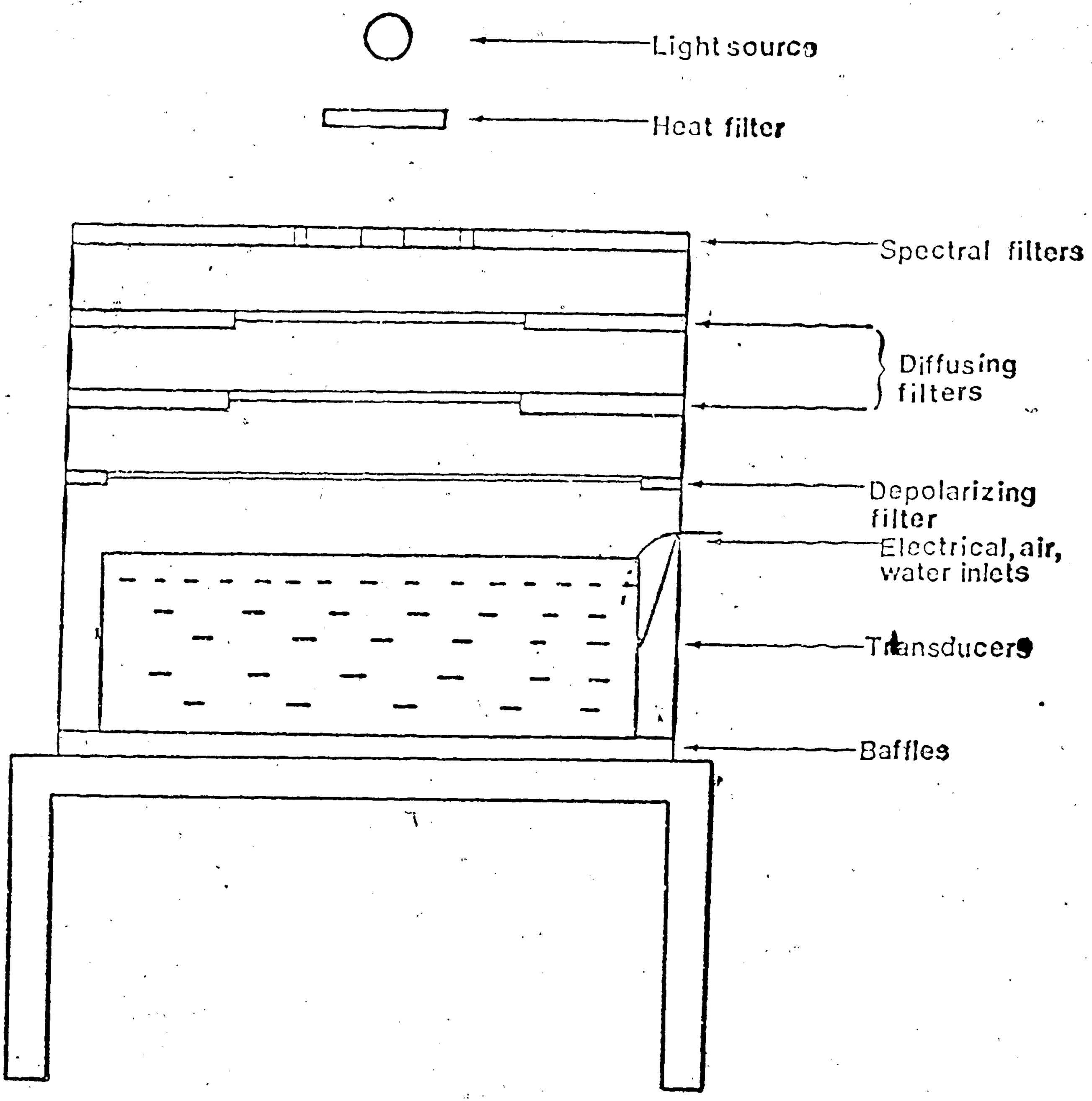
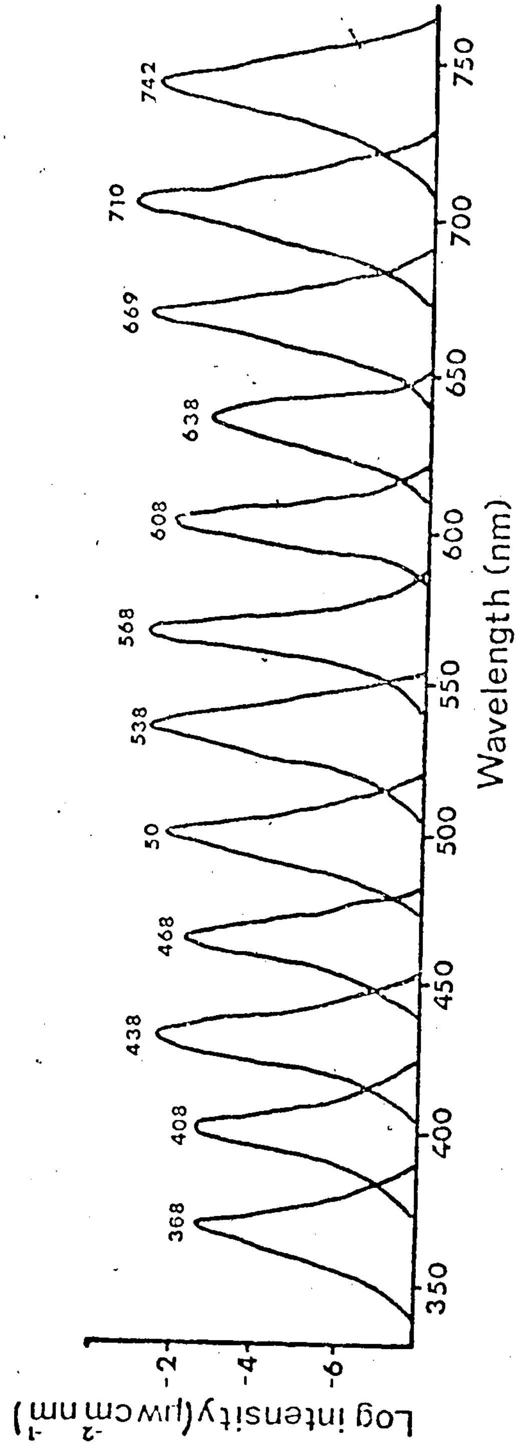

Light source
Heat filter
Spectral filters
Diffusing filters
Depolarizing filter
Electrical, air, water inlets
Transducers
Baffles

Fig. 30. Transmission spectra of filters used in entrainment and action spectra determinations. The peak spectral value is listed above the transmission curve of each filter.


368
408
438
468
50
538
568
608
638
669
710
742
350
400
450
500
550
600
650
700
750
Wavelength (nm)
-2
-4
-6
Log intensity (μw cm^{-2} nm^{-1})

the spectral energies impinging on the tank. The filters transmitted spectral bands (±10 nm) rather than single wavelengths. Spectral energy measurements took into account the emission spectra of the source, optics of the system, any stray light present, and transmission of the diffusing filters. They were made by placing the optic fibers sensing head at various points below the surface of the water. When similar (±5%) energy values were obtained at various positions in the tank the light distribution was considered to be uniform. The light distribution obtained in the tank was similar to that present under simulated twilights.

In a preliminary determination single *C. plumbeus* (n=5) were held under DD until arrythmic activity was evident (5-7 days). Then, determinations were made of the energy and/or photon threshold required for the initiation of induction of changes in activity. The alterations in the amplitude and level of activity were considered as being equivalent to the transitions in activity that occurred at the beginning of LD entrained locomotion. Short pulses of light (15-30 sec) were applied at random times and the activity of chub was recorded. Light intensity was increased until a detectable activity response was recorded (Fig. 31). Activity changes were expressed relative to the alterations seen at LD transitions. These procedures were then repeated at various times.

(ii) Results

The results obtained are shown in Fig. 32A,B. Maximum alterations in activity or induction of activity were obtained with 439, 468, 568 and 638 nm spectral bands. For each spectral band there was a preferred

Fig. 31. An example of the inductive or activity altering effect of a 30 sec light pulse, I (4.0×10^{-6} μw cm^{-2} 438 nm^{-1} incident spectral energy; 10°C) represented by (---), on the locomotory behaviour of a single *Couesius plumbeus* held under DD. The effects of a non-inductive pulse, N.I. (1.3×10^{-7} w cm^{-2} 438 nm^{-1} incident spectral energy) are provided for comparison. Activity is expressed in relative units. The amount of activity displayed by *C. plumbeus* at the onset of maximal activity under an LD or LD+t photoperiod is considered as 100%.

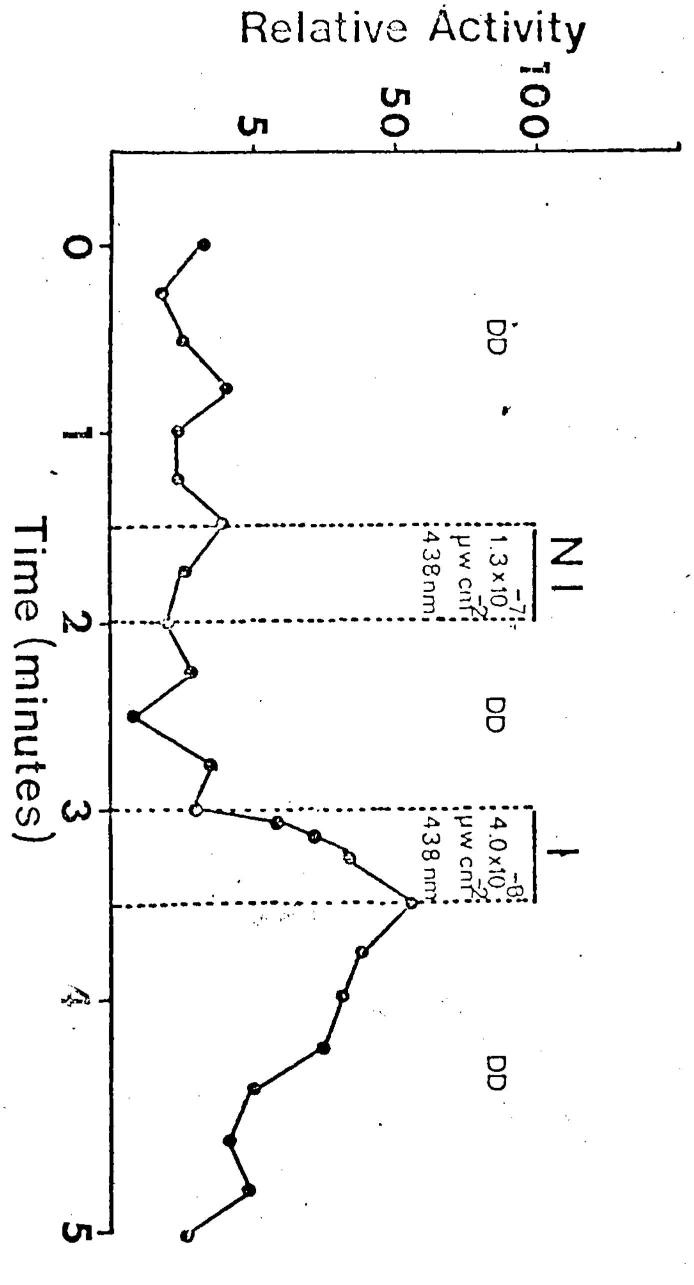

Relative Activity
100
50
5
0
1
2
3
4
5
Time (minutes)
DD
NI
1.3x10-7
µw cm-2
438nm
DD
I
4.0x10-8
µw cm-2
438 nm
DD

Fig. 32. A) Alteration or induction of activity in single *Couesius plumbeus* plotted as a function of the rate of change of initial spectral energy. Activity was measured relative to the maximal activity alterations of fish under LD and LD+t photoperiods. The latter were considered 100%.

Fish (n=10.3) were maintained at different initial light energies, listed in 32B, and then exposed to 15-30 curvilinear pulses of spectral energy change (μw cm^{-2} sec^{-1}). Experiments were conducted at random times of day and different rates of energy change. Each point represents the mean of four measurements.

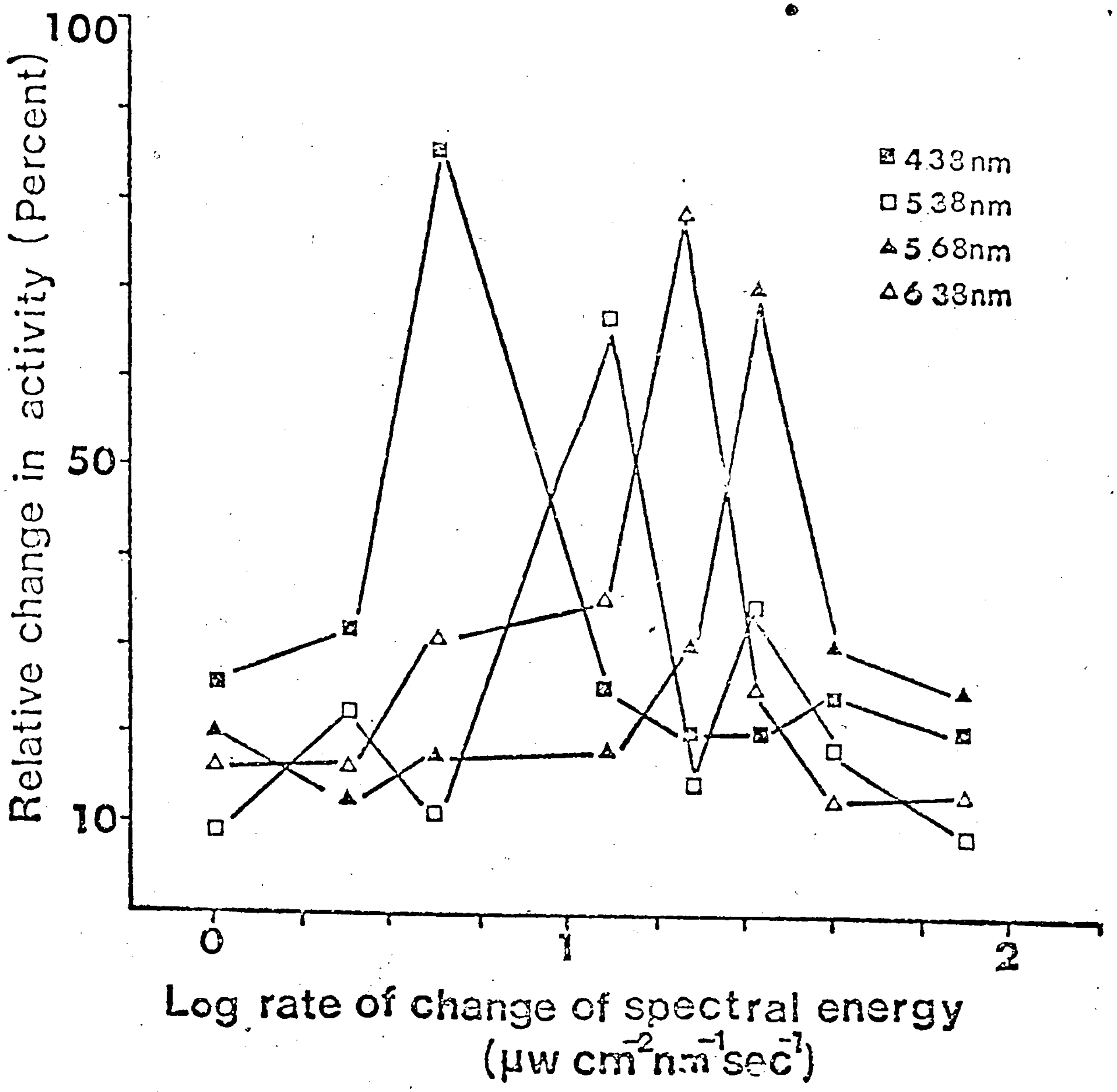
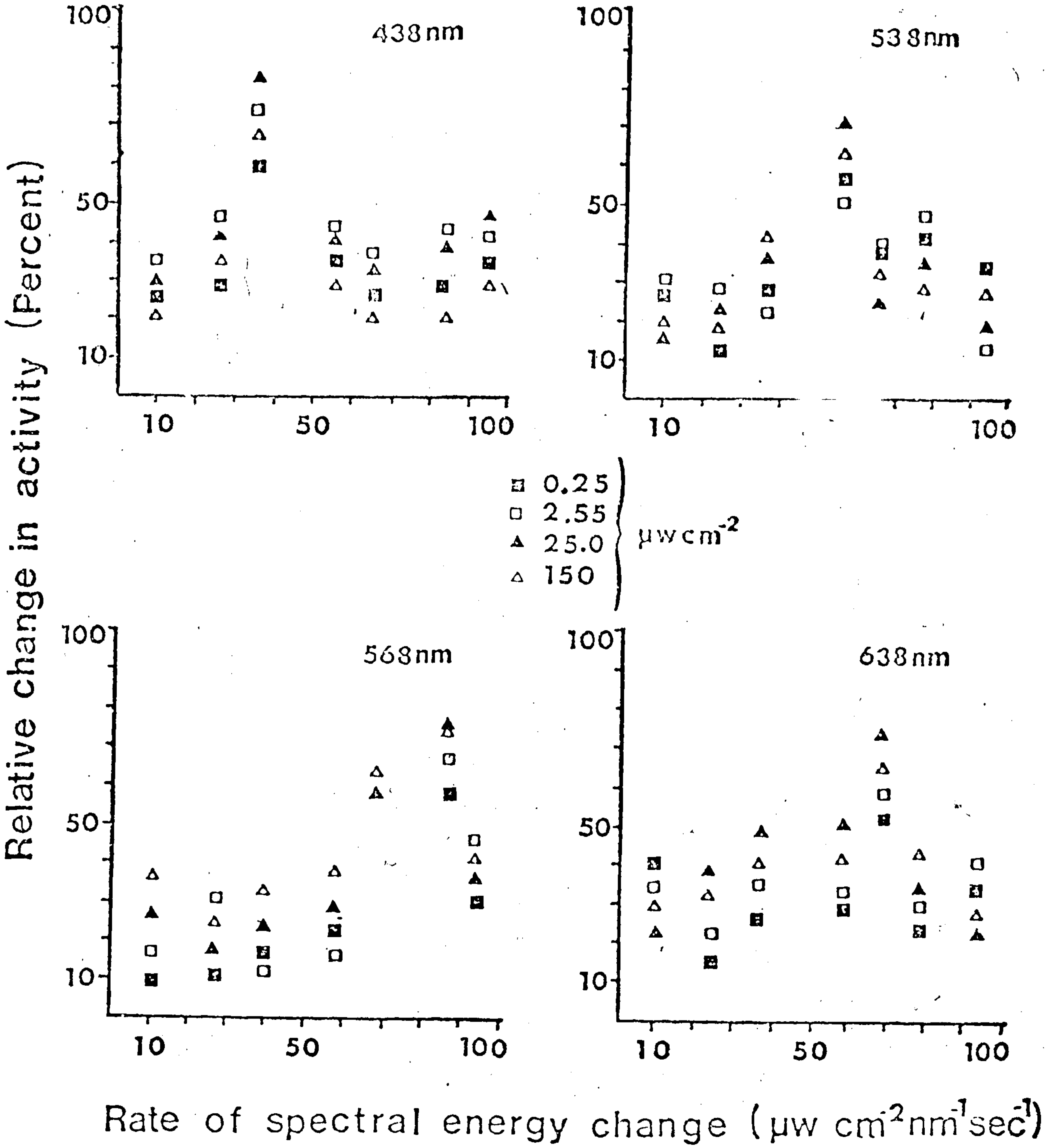

100
50
10
Relative change in activity (Percent)
0
1
2
Log rate of change of spectral energy
(µw cm^{-2}nm^{-1}sec^{-1})
▨ 438nm
□ 538nm
▲ 568nm
△ 638nm

Fig. 32. B) Alterations or induction of activity in single *C. plumbeus* (n=39) as a function of the initial spectral energy and its subsequent rate of change. Criteria used for analysis are described in 43A.


438nm
538nm
568nm
638nm
100
50
10
10
50
100
0.25
2.55
25.0
150
μwcm^{-2}
Relative change in activity (Percent)
Rate of spectral energy change (μw cm^{-2}nm^{-1}sec^{-1})

rate of energy change (Fig. 32A) that was independent of the initial energy levels (Fig. 32B).(F test $p < 0.05$). In this analysis only energy levels normally occurring during twilight transitions are considered. These preferred rates of change correspond to the values that were found at the beginning of activity under LD and LD+t photoperiods (see Tables 6, 7). Experiments were repeated at various times of the year.

D. Summary of Results

From the results of this chapter one can conclude that:

1) twilight, dawn and, to a lesser extent, dusk are required for the determination of seasonal changes in the entrainment of the activity of *C. plumbeus*;

2) the phasing of the beginning of activity (Ψ_{onset}) is dependent on specific characteristics of dawn. Ψ_{onset} has a bimodal annual pattern that is consistent with the duration of twilight (dawn) and changes in its sub-components. The end of activity (Ψ_{offset}) is determined by the total duration of photoperiod, rather than any specific features of dusk;

3) Ψ_{onset} is determined by specific rates of change of spectral energy, and spectral ratios that are found in dawn. Onset is not directly dependent on the absolute intensity of illumination.

E. Discussion

Studies with birds, mammals, and plants have shown that the following 6 factors (not necessarily in order of importance) are the major determinants of the phase relationships of circadian rhythms

(Aschoff 1969; Pittendrigh 1974; Daan and Aschoff 1975):

1) light-dark ratio (intensity or duration)
2) intensity of illumination
3) duration of twilight
4) other environmental factors in addition to photoperiod
5) physiological and behavioural state of the organism
6) natural free-running period (T) of the organism.

The results of the present study comparing the activity of *C. plumbeus* held under photoperiods with and without twilights, LD+t and LD respectively, while keeping other environmental factors or cues constant, conclusively showed that twilights were significant determinants of the entrained state. This agrees, in principle, with the previous laboratory findings for mammals and birds by Kavanau *et al.* (*loc. cit.*), Wever (1967), Daan and Aschoff (1975), Kenagy (1976), and with field observations for fishes (Hobson 1972, Emery 1973). This study also shows that these differences in entrainment between LD and LD+t are seasonally variable, depending on specific twilight characteristics.

Under natural outdoor laboratory simulated photoperiods, LD and LD+t, *C. plumbeus* displayed a bimodal diel activity pattern. When twilights were present (LD+t), the beginning of activity occurred during dawn, while the end of activity occurred during dusk. Under LD photoperiods the onset of activity preceded the dark-light change, while the termination of activity occurred at the light-dark transition. The latter can be described as an exogenous shock reaction and as a masking effect, a characteristic response of most fishes experiencing sudden changes in the intensity of light (Davis 1962). A masking effect is considered as a direct action of the light-dark cycle (transition) either causing or, as in this case, suppressing the rhythm rather than functioning as an entraining agent (Aschoff 1960, Hoffman 1969). The former response can be considered as an anticipatory onset, a characteristic that is indicative of an endogenous circadian system (Pittendrigh 1974).

Activity transitions occurring during twilights have also been assumed to be the result of masking effects. However, the presence of seasonal changes in activity relative to light (see Fig. 22) indicates that the beginning and end of activity reflect definite phase points of an endogenous oscillator rather than externally evoked responses.

Entrainment of circadian rhythms by photoperiod, LD+t, can involve two different routes of zeitgeber action: (i) proportional (parametric) or integral action — the continuous or intermittent effect(s) of the duration or intensity of illumination, and (ii) differential (non-parametric) action — the actual light-dark and dark-light transition i.e. twilight component (Aschoff 1969).

Although synchronization by the action of twilights or LD changes by definition implies non-parametric (transition) entrainment, it is still possible for parametric (proportional) effects to act on the system. Pohl (1976) examined effects of light on circadian activity rhythms of birds and found a relationship between light intensity and the extent and duration of changes in the midpoint phase angle under low light levels (lux).

This seasonal study revealed that daylight and twilight components of photoperiod have different roles in the synchronization of the activity of *C. plumbeus*. The timing of the beginning of activity relative to sunrise, Ψ_{onset}, is cued by the duration, rate of change and specific rates of spectral energy change and spectral ratios (see Tables 6, 7 and Figs. 27, 28, 32). These spectral factors follow a seasonal pattern in their occurrence that is coincident with changes in the duration of twilight. This results in Ψ_{onset} having a bimodal annual pattern consistent with the duration of twilight and its seasonal rate of change. The end of activity, Ψ_{offset}, followed a unimodal pattern consistent with annual changes in daylength and the absolute intensity of illumination. Only one other study of seasonal changes in daily activity has found a bimodal, twilight cued or related pattern for onset (dusk cued) and a unimodal pattern for offset (dawn cued) (Kenagy 1976). That study was with nocturnally active kangaroo rats. Few inferences can be directly drawn to the results obtained with *C. plumbeus*. However, it appears that the relative importance of dawn or dusk as Zeitgebers depends on which of the twilight transitions phases the beginning of activity; that is, whether the activity of a diurnal or nocturnal animal is being examined.

Seasonal changes in the phasing of the beginning and the end of activity were first described from annual changes in bird activity. Aschoff (1960) proposed that daylength, light intensity, and light-dark ratios were the main determinants of seasonal phase relationships, while Wever (1967) suggested that twilight duration was the main determining factor. He proposed that: the longer the twilight period the earlier the occurrence of activity onset relative to twilight, and the greater are the phase angle differences with changing L:D ratios. As an extension to these general findings it was suggested that the greatest relative phase-delay should occur at the equinoxes, and that an intermediate phase angle should be found at the opposite solstice to that at which maximum advance occurs. Both Aschoff's and Wever's suggestions have been incorporated into a joint daylight-length/twilight-duration model of entrainment (Aschoff 1969).

In a recent review of photoperiodic entrainment Daan and Aschoff (1975) listed some features of the timing of activity that they considered valid for all animals. These included the following:

1) The beginning of activity (Ψ_{onset}) is generally more precise than the end (Ψ_{offset}).

2) Under increasing photoperiod durations activity of *diurnal* animals starts earlier in 'local time' but later relative to sunrise (Ψ_{onset} is smaller) and terminates later in local time but earlier relative to sunset (Ψ_{offset} is smaller).

3) The beginning and end of activity are more precise when they occur during civil twilights.

They considered the increased precision of Ψ to be a function of the greater rate of change of light intensity found in twilight.

Daan and Aschoff (1975) indicated that there were significant differences between phase values obtained from low and high latitudes. However, they also showed that there were differences between the timing of bird and mammal activity that restricted any extensive generalizing.

Figala and Müller (1972) conducted a preliminary examination of seasonal changes in the activity of a tropical fish, *Barbus partipantazona*, transported to the Arctic and found some similarities to the timing of bird and mammal activity. However, in view of the unnatural conditions that their fish were exposed to, few conclusions are possible from the study.

There is only partial agreement between these general theories for the timing of rhythms and the phasing of the activity of *C. plumbeus* obtained from the present study. Greatest advance in Ψ_{onset} occurred at the summer solstice, but the greatest delay was not at the winter solstice, but rather at the equinox at which twilight was the shortest. Discrepancies arise because the theoretical proposals are based on the tacit assumption of similar cues being used for the timing of onset and offset of activity. The present study showed that this clearly was not the case, twilight characteristics being used as the prime determinants of Ψ_{onset}. Utilization of a greater number of total cues for onset leads to the 'apparent' greater precision of the beginning of activity. This precision is primarily a factor of the greater number of cues used, these being less susceptible to stochastic environmental variations. The twilight components provide a consistent time-giving signal.

The response to specific rates of spectral energy change rather than absolute intensity is not consistent with the circadian entrainment generalizations of Daan and Aschoff (1975). However, these appraisals were obtained from the laboratory activity of birds and mammals and were expressed in photometric units. Their light values cannot be used as an accurate index of the true light characteristics.

From analytic studies with single spectral components it was found that almost all portions of the 'visible' spectrum (400-750 nm) had some modifying effect(s) on the activity of *C. plumbeus* (see Fig. 33). However, there were significant differences in the efficiency as well as photon and energy thresholds at the various spectral regions. The most effective inductive spectral values overlapped with the wavelengths whose rates of change of energy were correlated with the beginning of activity of *C. plumbeus* under LD+t (Ψ_{onset}). Specific rates of spectral energy change were the functional cues for inducing activity alterations, as well as the onset of entrained activity. The same preferred rates of energy change were used regardless of initial light energy, within the limits of twilight intensities. This permitted utilization of a number of constant time cues regardless of other photic modifications. Other photic and environmental factors could function to modify the initial timing of activity.

Demmelmeyer and Harrhaus (1972) and Kavanau and Rischer (1972) have shown that daily cycles of light of different colour temperature are capable of weakly synchronizing or coordinating finch and ground squirrel activity. Krull (1976a,b) suggested that slight oscillations in low spectral energies, coupled with changes in spectral composition or ratios, may act as Zeitgebers for Arctic animals. Thus the entrainment responses demonstrated for *C. plumbeus* may be indicative of a more widespread and general mechanism of entrainment.

Krüll (1976a) speculated that changes in far-red/red spectra ratios, in combination with slight intensity changes, are utilized as entraining cues by Arctic animals. These ratios retain their daily modifications in total energy or quantal levels. Seasonal changes in dawn and dusk FR/R signals are determined by geophysical parameters and are quite independent of intensity and other surface spectral modifications. These spectral alterations have the precision necessary for use as consistent photoperiodic cues. This constancy suggests that these spectral ratios are highly precise signals that could be widely used as entrainment cues. These results show that spectral ratios and rates of energy change have the ability to function as effective entrainment cues.

Utilization of selected spectral intensity changes and ratios provides a consistent non-parametric signal for timing of activity and its various behavioural constituents. Stochastic variations in the environment may disrupt some of these spectral cues but will not eliminate all of them, hence permitting consistent Ψ_{onset} determination. The end of activity is loosely entrained by, or follows, daylength, a factor which is subject to considerable environmental fluctuation. This permits significant flexibility in the termination of activity in response to stochastic variations in the environment.

Chapter V

THE FREE-RUNNING PERIOD AND CIRCADIAN ACTIVITY

A. Introduction

Under constant photoperiod conditions (DD or LL) birds and mammals display endogenous circadian rhythms of locomotory activity that perisist for prolonged periods (100-500 cycles) in the free-running state (Eskin 1971; Hoffman 1971). The constant periods and activity forms displayed in these long runs are described as steady-state values. The free-running period (FRP) values of nocturnal animals (birds and mammals) under DD are generally, but not always, less than 24 hrs while those of diurnal animals are usually greater than 24 hrs. The presence of crepuscular components can obscure these relationships and make any consistent and conclusive generalizations difficult to produce (Kavanau 1969). Increasing or decreasing FRP values occurring during the transition from one entrained state to another are defined as transient period values.

Endogenous tau values that are influenced by the immediate photoperiodic entrainment history of an animal are known as after effects. Implicit in this definition are all tau values that are found immediately (1-20 cycles) after imposition of constant conditions. In nocturnal mammals, the only group examined in detail, the initial T value appears to be a direct function of the duration of the previous dark or light period (Pittendrigh and Daan 1976b).

Although the circadian rhythm of activity is a persistent property of an endogenous oscillator system, the expression of the pattern and period of activity can be influenced by exogenous non-entraining stimuli. These can directly suppress or accentuate some parts of the circadian pattern or alter the overall periodicity. For example, continuous light (LL) and continuous dark (DD) have different effects on the period length and subsequent fade-out of circadian rhythms. It has also been shown that the FRP depends on the intensity of illumination. This relationship is expressed as part of a generalization known as the 'circadian' or Aschoff's rule (Aschoff 1960, 1965). According to this proposition the spontaneous frequency (1/period), the ratio of activity to rest time, and total amount of activity should increase with greater light intensity in light-active animals and decrease in dark-active animals. The extent and duration of activity in the 'active portion' of the rhythm also shows a similar relationship to the intensity of constant illumination. Kavanau (1969) has shown that for many mammals the circadian rule and proportional effects of light apply only in the middle ranges of light intensity that are found in twilights.

A synthetic appraisal of the possible functions and roles of circadian periods in the generation and entrainment of the activity of nocturnal animals is provided by Pittendrigh and Daan (1976a,b,c) and Daan and Pittendrigh (1976a,b). They put forward that the attainment of a stable entrained state bears a definite relationship to the FRP. They also proposed that the entrained state (photoperiodic history), measured as a function of Ψ, determines the immediate tau values. How these relationships arise is a matter for further consideration.

All the properties described for vertebrate circadian rhythms have been derived from studies with birds and mammals. There have been limited investigations of fish circadian rhythms. Locomotory rhythms have been most extensively examined in the cyprinid *Leucaspius delineatus* (Siegmund 1969; Siegmund and Wolff 1972a,b,c, 1973) and several other northern European and Arctic species of fishes (Müller 1970, 1973; Eriksson 1973). Reviews of the more tangible studies of endogenous locomotory or behavioural rhythms of fish are provided by Schwassman (1971a), Richardson and McCleave (1974), and Beitinger (1975). However, only limited descriptions and interpretations of circadian rhythms in fish were made in all those studies.

The objectives of this chapter are twofold: firstly, to describe and determine basic circadian parameters of a temperate freshwater fish; secondly, to determine if there are any relationships between the characteristics of entraining photoperiods and tau values; more specifically, to determine if twilight has any effects on the values of the subsequent free-running period. /This latter examination was done in conjunction with the analyses of the roles of twilight in the entrainment of activity (see Chapter IV).

B. Methods and Results

(i) General activity

Fish that had been kept (entrained) under LD or LD+t laboratory conditions, as well as individuals that were obtained from the natural conditions of the river, were used for the tau determinations. The same entraining conditions and fish were used in the analyses of the timing of the beginning and end of activity (Ψ's). The exact experimental procedures used for entrainment analyses are described in Chapter IV. The fish sampling sequences are listed in Tables 8 and 9. Period determinations of any one fish were not repeated until a steady state of entrainment was obtained after return to laboratory photoperiods. This generally required 1-2 months. The number of times tau was determined was essentially a compromise between the requirements of phase and period calculations.

All of the period measurements were repeated for at least a second annual photoperiod cycle. These latter measurements were carried out in conjunction with replications of Ψ measurements and investigations of more specific aspects of entrainment.

All healthy fish (n=104) held under DD or LL evinced free-running rhythms of locomotory activity for at least 3-7 cycles. The endogenous patterns lost synchrony with the previous photoperiods and extinguished to an apparent mean level of non-stationary activity (Fig. 33). Accurate period values were most readily obtainable from the spectral transforms and periodograms. Under constant conditions periods ranged from 24.5 to 28.0 hrs (± 0.4).

All of the individuals entrained under LD+t photoperiods (n=54) had repeatable, significant seasonal variations in their free-running period values. The general activity patterns, aside from sub-component or 'bout' composition (see Appendix III), were the same throughout the year. At lower temperatures (0°-4°C), which were coincident with the shorter photoperiods of winter, the amplitude of activity was reduced but the form was not affected. The free-running circadian activity

Table 8. Monthly free-running activity period (Tau) values of single *Couesius plumbeus* kept under simulated laboratory photoperiods that include twilights (LD+t).

Fish #	Mean Period Length (hours)[1]											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
C6	26.1 ±.3							25.9 ±.4				
C7		25.8 ±.2							26.4 ±.5			
C12	26.4 ±.5		25.3 ±.4				25.6 ±.2					
C15				24.9 ±.4					25.8 ±.4			
C18					24.7 ±.3					26.7 ±.6		
C22		26.6 ±.6				24.9 ±.3					27.6 ±.8	
C27			24.7 ±.3				25.2 ±.3					26.0 ±.3
C29	26.5 ±.4							25.7 ±.3				
C32					25.3 ±.4				26.4 ±.3			
C38			25.1 ±.3							26.9 ±.3		
C40				25.6 ±.4							26.5 ±.8	
C41						24.7 ±.4						26.4 ±.5
Mean Period	26.3 ±.4	26.1 ±.4	25.1 ±.3	25.3 ±.4	25.0 ±.3	24.8 ±.4	25.4 ±.3	25.8 ±.4	26.2 ±.4	26.8 ±.3	27.1 ±.8	26.4 ±.4

[1] The plus and minus (±) values are 95% confidence intervals.

Note: At selected intervals fish were placed under constant dark (DD) and Tau was determined over 5-7 cycles. Values are for 1975-1976. Age, sex, and entrained activity characteristics are given in Table 3.

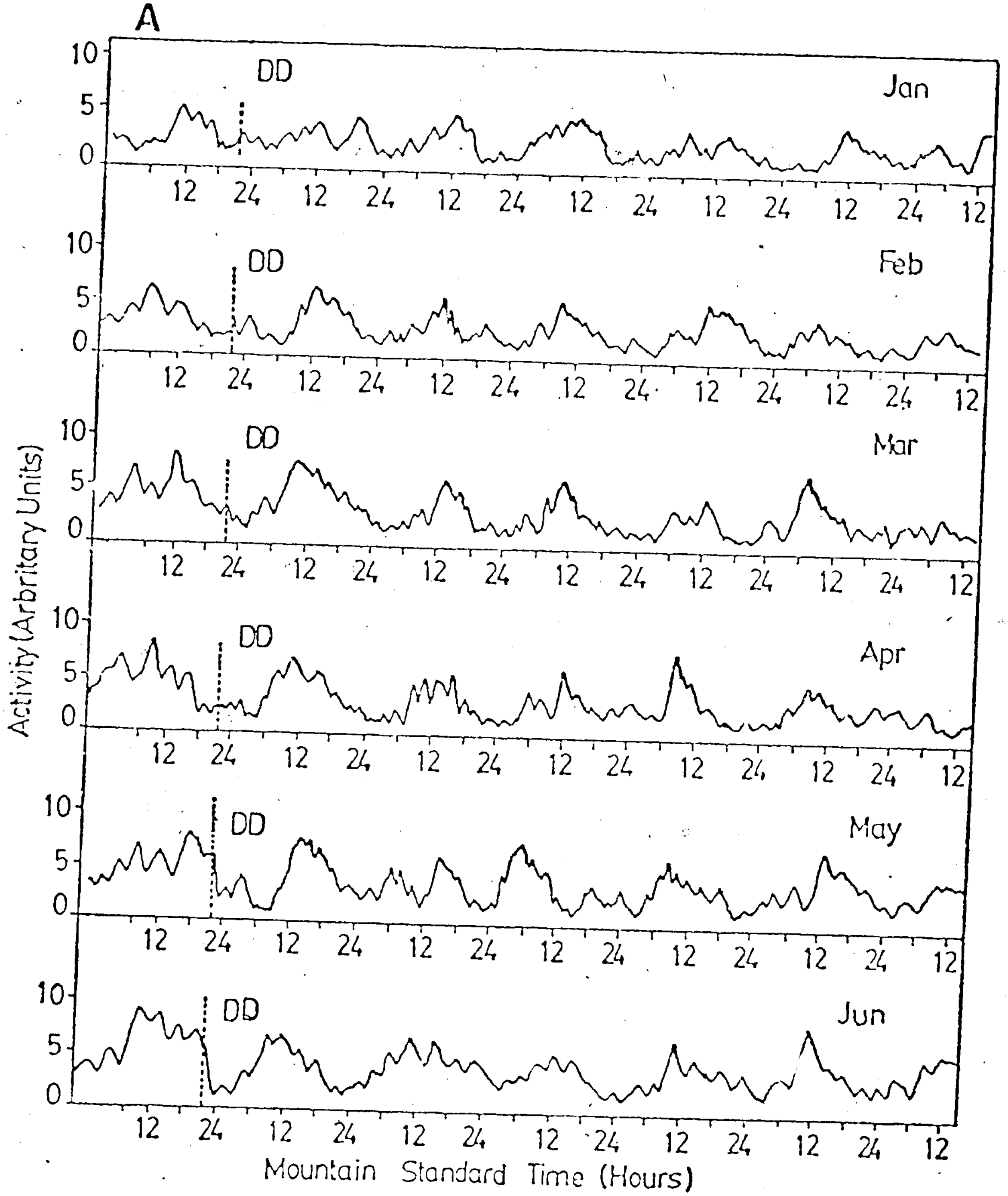
Table 9. Monthly free-running activity period (Tau) values of single chub (*Couesius plumbeus*) kept under laboratory photoperiods without twilights (LD).

Fish #	Mean Period Length[1] (Tau) (hours)											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
C5	25.4 ±.7				25.8 ±.6							
C8		25.2 ±.9					29.5 ±.5					
C9			25.9 ±.1					26.3 ±.2				
C16				27.0 ±.3						26.2 ±.7		
C23					26.7 ±.4				24.8 ±.3			
C25						25.7 ±.8					27.4 ±.7	
C28							24.9 ±.6					25.9 ±.8
C30	27.9 ±.6							24.8 ±.3				
C33		25.9 ±.7							25.8 ±.4			
C34				25.7 ±.3						27.7 ±.9		
C35						27.7 ±.3					23.7 ±.7	
C36			24.7 ±.6									25.6 ±.8
Mean Period	26.65 ±.3	25.55 ±.8	25.3 ±.4	26.35 ±.3	26.29 ±.5	26.70 ±.5	26.2 ±.6	25.55 ±.3	25.3 ±.4	26.95 ±.8	25.55 ±.7	25.75 ±.8

[1] The plus and minus values (±) are 95% confidence intervals.

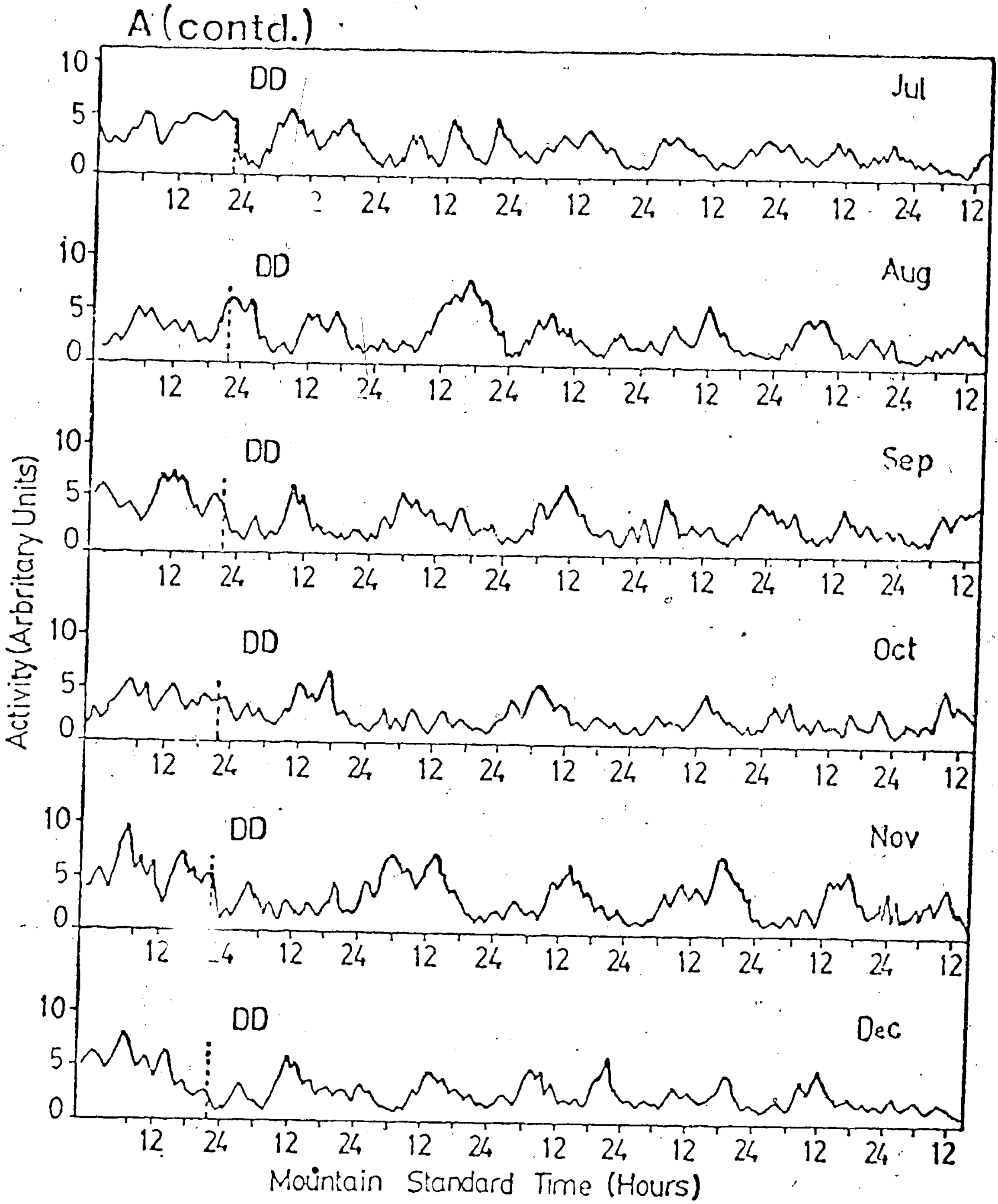
Note: At selected intervals fish were placed under constant dark (DD) and Tau was determined over 5-7 cycles. Values are for 1975. Age, and entrained activity characteristics are given in Table 5.

Fig. 33. Example of the monthly free-running locomotory activity of *C. plumbeus* under DD that had been previously kept under seasonal photoperiods: A) with twilights (LD+t); B) without twilights (LD).


A
Activity (Arbitrary Units)
10
5
0
DD
Jan
Feb
Mar
Apr
May
Jun
12
24
Mountain Standard Time (Hours)

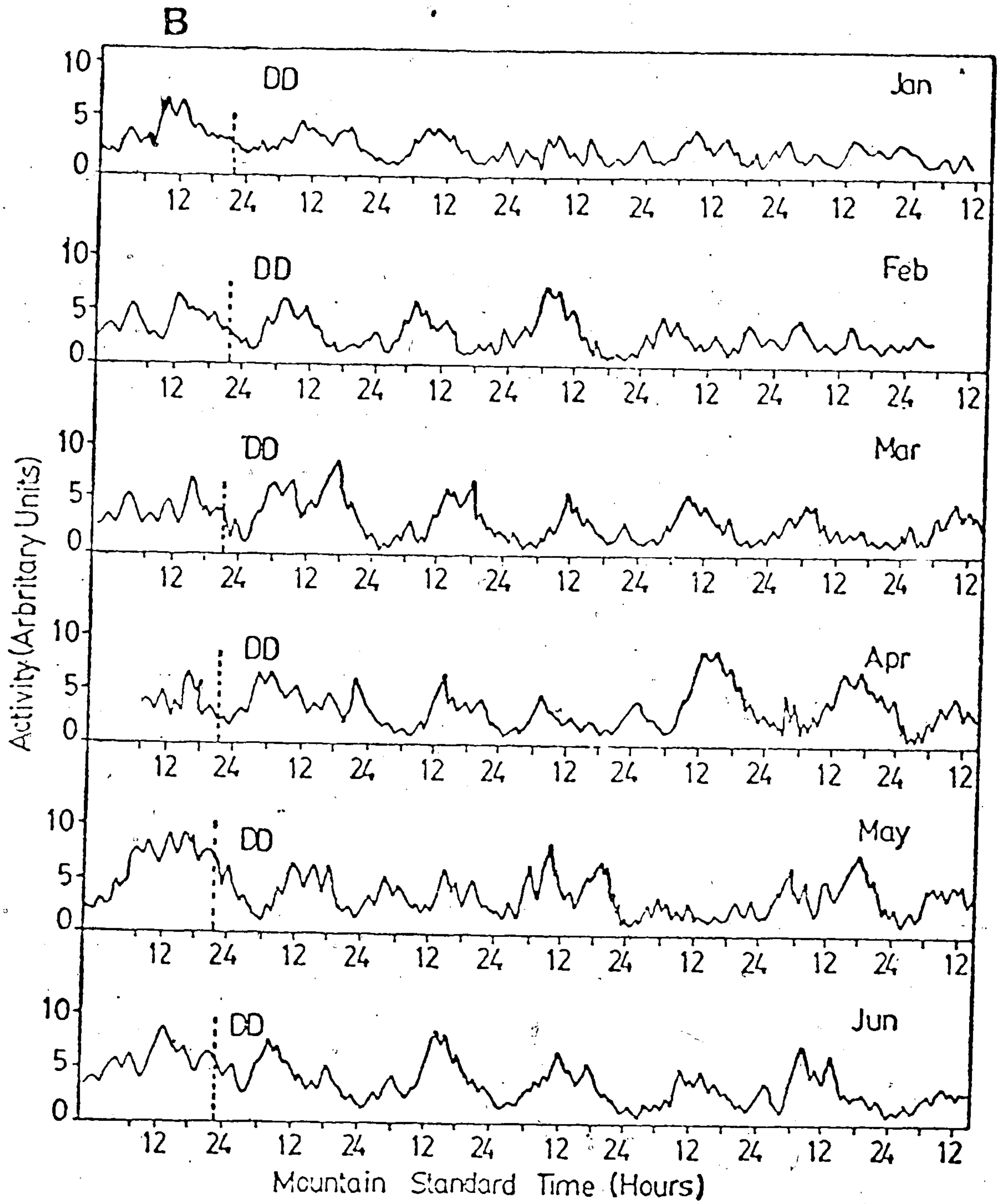
(Cont'd)

Fig. 33 (Cont'd)


A (contd.)
Activity (Arbritary Units)
10
5
0
DD
Jul
Aug
Sep
Oct
Nov
Dec
12
24
Mountain Standard Time (Hours)

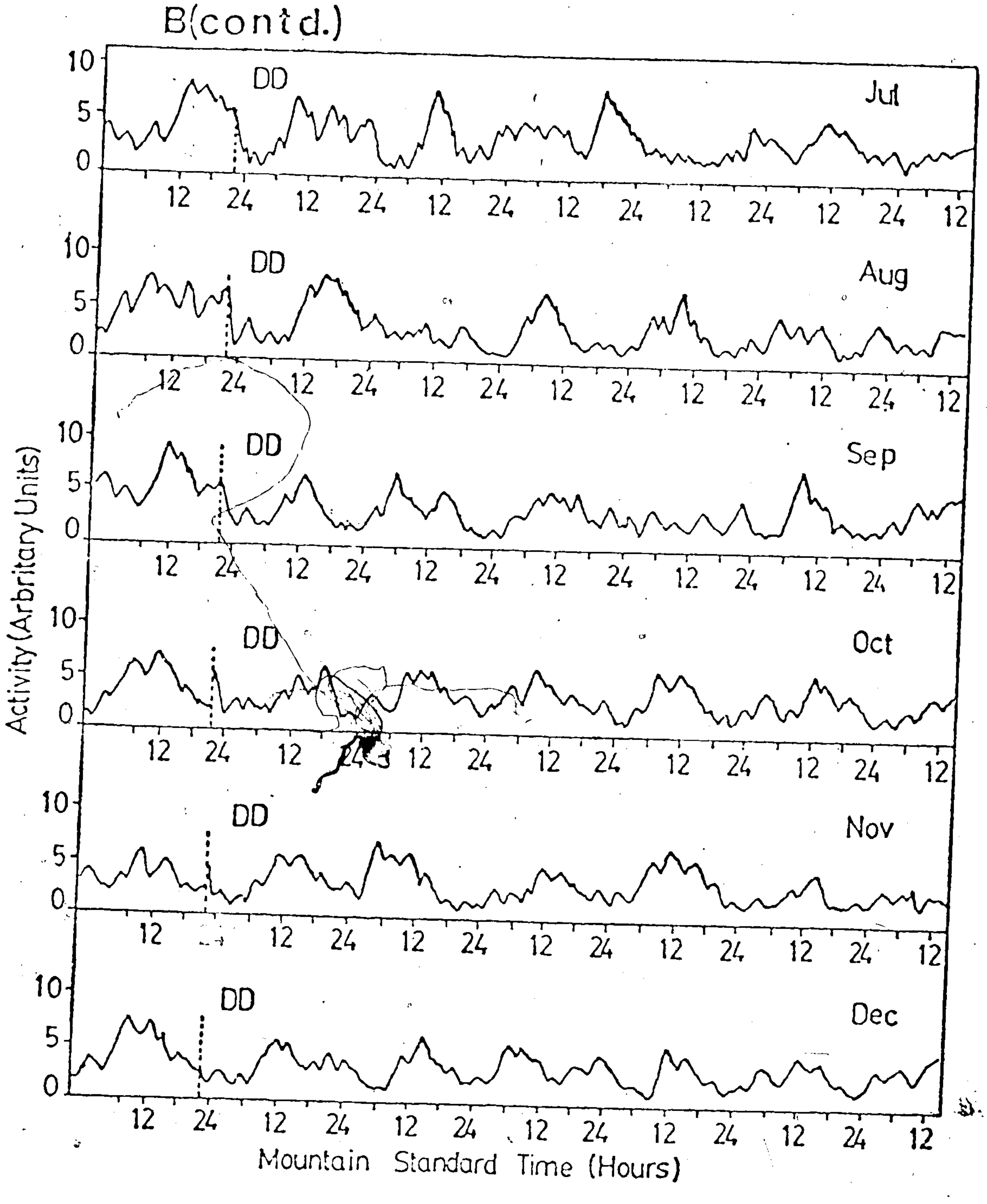
(Cont'd)

Fig. 33 (Cont'd)


B
Activity (Arbitrary Units)
10
5
0
DD
Jan
Feb
Mar
Apr
May
Jun
12
24
Mountain Standard Time (Hours)

(Cont'd)

Fig. 33 (Cont'd)


B(contd.)
DD
Jul
Aug
Sep
Oct
Nov
Dec
10
5
0
12
24
Activity (Arbritary Units)
Mountain Standard Time (Hours)

(Cont'd)

pattern was essentially a continuation of the entrained amplitude and sub-components.

C. plumbeus had a significantly ($p < 0.001$ Mann-Whitney U test) shorter period during the summer than the winter (24.7 hrs as compared to 27.1 hrs) (see Fig. 34A). The lengths of the free-running activity periods of *C. plumbeus* were significantly and positively correlated ($p > 0.05$; $r = +0.73$) with the duration of photoperiod from LD+t (see Fig. 35A), though no significant relationships to twilight were evident (see Fig. 35C).

Period availability increased immediately before extinction of the rhythm (see Fig. 33). However, the degree of variation was consistent within similar temporal and environmental conditions, thus allowing comparisons to be made with either individual fish or pooled samples. Precision of T (a reciprocal of standard error) generally increased as the FRP approached 24 hrs. That is, there was a greater cycle-to-cycle stability in the FRP as it approached the exogenous periodicity of 24 hrs (see Fig. 33). A Kendall's partial correlation of $r = 0.42$ ($p > 0.4$) revealed that there were no interactions or relationships between precision and time of year. This correlation value also indicated that a major portion of the annual variation in precision could be explained by, or attributed to, changes in tau. No significant relationship was detected between tau and Ψ_{onset}.

Significant seasonal differences in FRP lengths were recorded from *C. plumbeus* (n=21) that were caught at various times of the year and then placed under DD (Table 10). The extent and range of taus were consistent with the values found under prolonged LD+t entrainment (see Table 10).

Table 10. Free-running period lengths (Tau) of *Couesius plumbeus* from natural conditions.

Month	Tau[1] (hours)		
	1974	1975	1976
Jan.	26.8 ± .5		25.9 ± .7
Feb.	26.2 ± .4	25.1 ± .6	
Mar.	24.6 ± .3		25.2 ± .3
Apr.	24.7 ± .3		
May	24.7 ± .5		24.9 ± .6
June	24.8 ± .6	24.9 ± .7	
July	25.0 ± .4		
Aug.	26.2 ± .6	26.0 ± .7	
Sept.	26.4 ± .4		25.9 ± .5
Oct.	26.3 ± .4	26.9 ± .4	
Nov.	27.0 ± .4		
Dec.	26.7 ± .2	27.0 ± .3	

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[1] The plus and minus values (±) are 95% confidence intervals.

Note: Fish were caught at selected monthly intervals and immediately placed under constant conditions (DD). Tau is based on 5-7 cycles.

C. phoebus (n=47) that were entrained to laboratory LD photoperiods, set either to sunset-sunrise or civil twilights, did not display any significant seasonal changes in their tau values or relationships to photoperiod (Figs. 34, 35, see Table 8). An increase in total variance (instability) coupled with a reduced tau range, relative to values from LD+t, caused or led to insignificant annual differences in period values. The general activity pattern seen in these records, aside from mean 'bout' lengths (see Appendix III) were similar to those obtained from LD+t photoperiod histories.

The presence or absence of twilight was the major difference between LD and LD+t photoperiods. The total durations of light (photofractions) were of equivalent length. However, the relationships between tau and the total photofraction of LD and LD+t differed significantly. The plot of LD+t against tau yielded a significant regression and correlation ($r = +0.73$; $p < 0.005$, Pearson's Z correlation). The LD graph (see Fig. 37B) revealed no significant dependencies ($p > 0.25$). At photoperiods of equivalent length (LD set to civil twilight) there is some overlap of the tau values from LD and LD+t. This overlap is probably the result of the increased variance of the FRP values from LD entrainment histories.

Temperature regimes were the same under all photoperiods thus eliminating the possibility that seasonal temperature differences were the cause of annual fluctuations in tau.

Thus the basic conclusion emerging from this portion of the study is: Photoperiods with twilights (LD+t) have a significant effect on the free-running period. When twilights are present in the previous

Fig. 34. A) Seasonal changes in tau values of *C. plumbeus* entrained under simulated natural LD+t photoperiods and then placed under DD. Vertical bars denote one standard error. (For each month n=12).

B) Seasonal changes in tau values of *C. plumbeus* entrained under seasonal LD photoperiods and then placed under DD. Vertical bars denote one standard error. (For each month n=12).

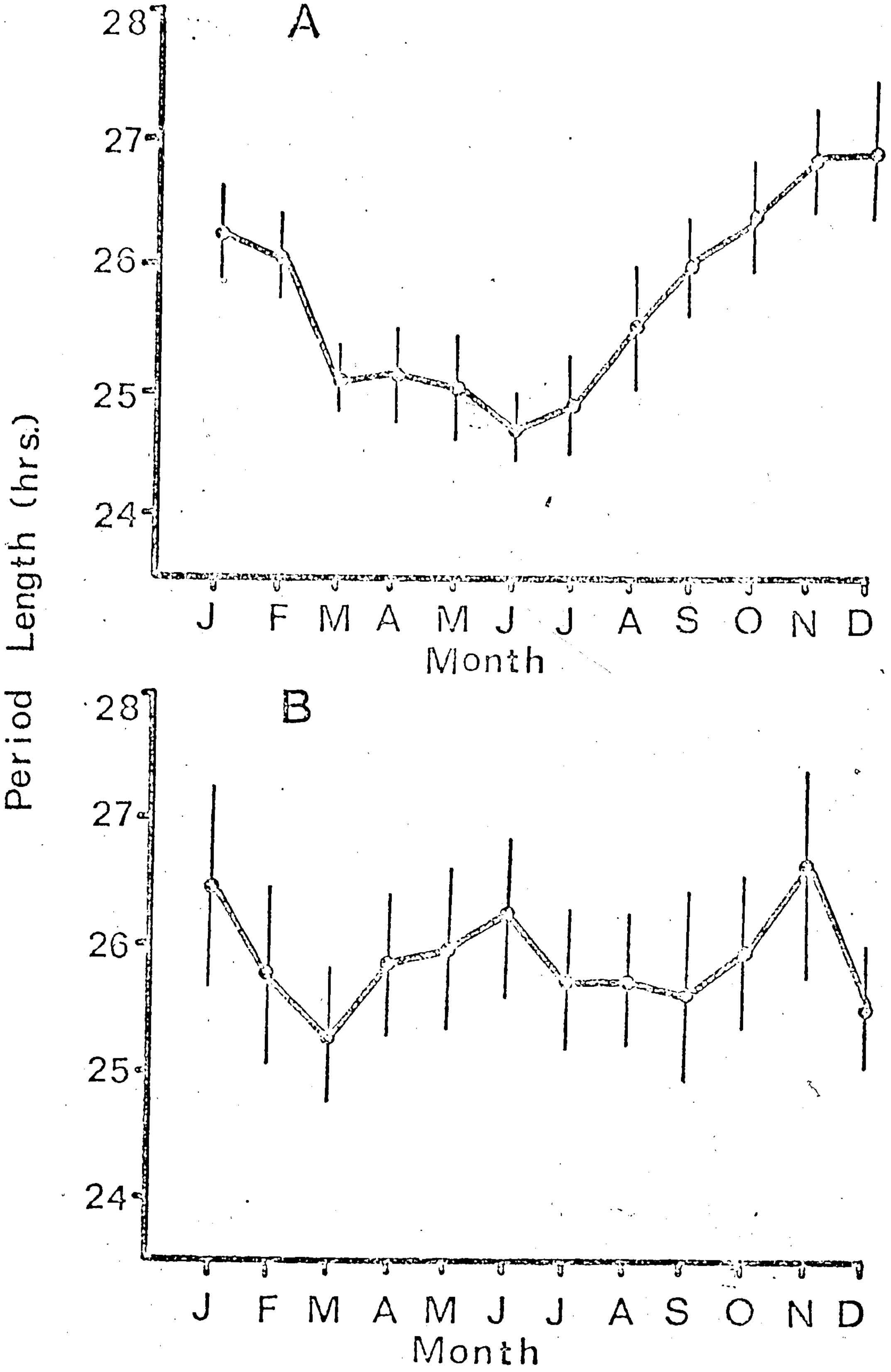
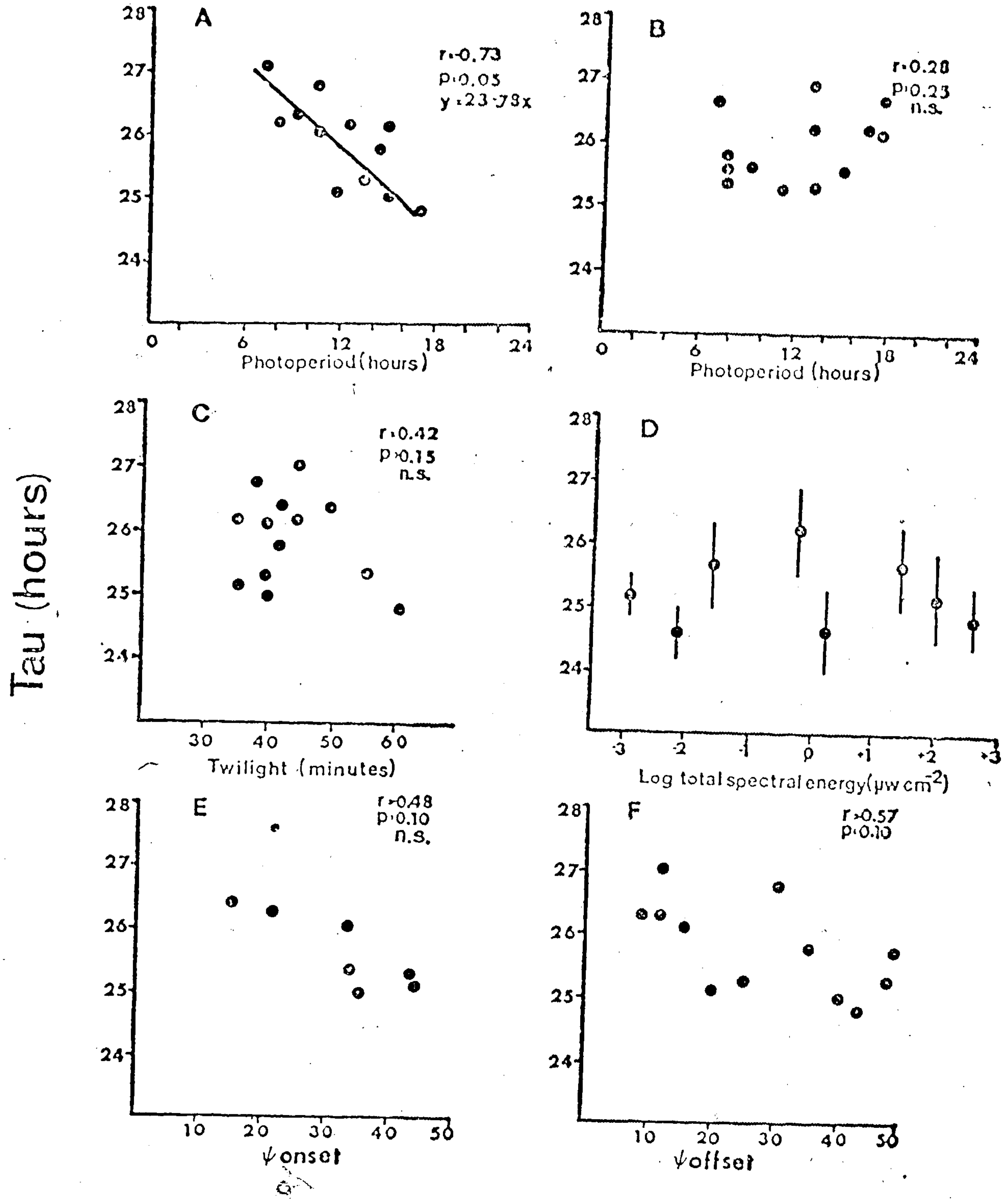

A
B
Period Length (hrs.)
28
27
26
25
24
J F M A M J J A S O N D
Month

Fig. 35. Relationships between tau (T) values of *Couesius plumbeus* and various parameters of previous entrainment history (LD or LD+t). All period measurements except (D) were made under DD.

	Previous entraining photoperiod	Parameter	Relationship
A)	LD+t	photoperiod	$p<0.05$ sig. ($r=0.73$)
B)	LD	photoperiod	$p>0.25$ n.s.
C)	LD+t	civil twilight	$p>0.15$ n.s.
D)	LD/LD+t	(LL) illumination intensity	$p>0.35$ n.s.
E)	LD+t	Ψ_{onset}	$p>0.15$ n.s.
F)	LD+t	Ψ_{offset}	$p=0.50$ n.s.


Tau (hours)
A
r=-0.73
p<0.05
y=23-.73x
28
27
26
25
24
0
6
12
18
24
Photoperiod (hours)
B
r=0.28
p<0.23
n.s.
28
27
26
25
24
0
6
12
18
24
Photoperiod (hours)
C
r=0.42
p>0.15
n.s.
28
27
26
25
24
30
40
50
60
Twilight (minutes)
D
28
27
26
25
24
-3
-2
-1
0
+1
+2
+3
Log total spectral energy(μw cm^{-2})
E
r=-0.48
p<0.10
n.s.
28
27
26
25
24
10
20
30
40
50
ψonset
F
r=-0.57
p<0.10
28
27
26
25
24
10
20
30
40
50
ψoffset

entraining state there are significant annual variations in tau.

(ii) Effects of light intensity

At randomly selected times of the year tau values were determined from single *C. plumbeus* (n=5 for 12 determinations) held under different light intensities (LL 0.1 to 350 μw cm^{-2}). No consistent or significant relationships to intensity were found (see Fig. 35D). However, the presence of large variabilities in period values measured under higher light intensities mitigates evaluation of any exact relationships.

C. Discussion

It has been sta[illegible] locomotor activity is not a particularly good parameter [illegible] the determination and analysis of circadian rhythms of fish (Schwassman 1971a). This assumption is based on data that were obtained with relatively imprecise recording techniques and limited statistical analyses. There is also the implicit assumption that the locomotory activity of fish is a single behaviour comparable to the circadian activity parameters of endotherms. However, as was shown earlier in this study, the laboratory activity of *C. plumbeus* cannot be considered as being equivalent to a single behavioural entity such as mammalian wheel running or bird perch hopping.

The various behaviours comprising the activity of fish can have different time structures and properties. This leads to an overall circadian activity pattern that appears to be relatively imprecise and is subject to rapid disassociation and fade-out. These behavioural modifications, which are discussed more fully in the analysis of general activity, must be considered when comparing circadian rhythms of fish to those of other vertebrates.

The rapid extinction of the FRP, 3-7 cycles in most cases, precluded treating tau as a steady-state value. By definition all period values recorded immediately after imposition of constant conditions have to be considered as being, or reflecting, after-effects of the previous entrainment history (Pittendrigh and Daan 1976a). However, the relatively rapid extinction of the circadian period and absence of any direct evidence for the existence of steady-state tau values in no way detracts from the endogenous nature of the free-running rhythms. After-effects in tau values can be used to obtain an indication of the effects of the previous entrainment history on the endogenous circadian components. When steady-state values are reached all effects of the previous entrainment state are assumed to have been eliminated (Eskin 1971).

Results of the correlations of LD, LD+t, Ψ_{onset}, and Ψ_{offset} versus tau, along with seasonal fluctuations in tau and precision strongly indicate that dawn and dusk components of the light-dark cycles are important determinants of photoperiodic after-effects (see Figs. 32, 34). Seasonal variations in T were present in individuals caught and then immediately placed under DD. This indicates that seasonal variations in tau are present in wild fish and are not artifacts produced by prolonged laboratory confinement.

C. plumbeus that were kept under laboratory LD photoperiods did not display any significant seasonal changes in their FRP values. This apparent lack of an annual pattern in FRP can be interpreted as being due, at least partially, to the greater intra-period variability in tau from LD entrainment histories.

The presence of photoperiodic after-effects on the circadian activity of fish as exemplified by *C. plumbeus*, birds and mammals (Pittendrigh and Daan 1976a,b) indicates that there are similarities in the underlying circadian systems of all vertebrates. After-effects on tau have been postulated as reflecting the inherent lability of the circadian system.

The process of entrainment can be thought of as a succession of phase shifts that compensat for time differences between the period length of the endogenous cycle and that of the entraining cycle. Differences in tau can reflect the degree of adjustment required for entrainment under photoperiods of changing length. An increase in stability or precision of tau as it approaches 24 hrs may compensate for system and phase instability that is generated as a resonance condition as T=T=24 hrs is approached. Resonance is assumed by analogy with physical oscillators (Bünning 1973).

However, the present results also indicate that there are differences between the expression of circadian activity rhythms in fish and those of higher vertebrates. In birds and mammals the endogenous locomotory period appears to be a direct response to, or after-effect of, the preceding LD cycle. For instance, the FRP values of nocturnal hamsters are an inverse function of the duration of the light portion of the preceding entraining photoperiod (Pittendrigh and Daan 1976a). For diurnal animals these relationships would be shown by a decrease in tau values (Eskin 1971, Pittendrigh and Daan 1976b).

The seasonally invariant tau values obtained from fish entrained under LD photoperiod histories raises several potential problems.

There were no significant seasonal differences in tau values from LD entrainment histories and yet activity was consistently synchronized by these photoperiods. This suggests that compensatory alterations in tau may be experimental artifacts that are not essential for entrainment. However, it is also conceivable that the larger variability in period values of fish that were held under LD entrainment histories leads to an overlap with the T values necessary for complete entrainment. Occasional approximations of these 'ideal' conditions and phase relations may result in the weaker phase synchrony seen under LD photoperiods. In view of the significant role of twilights in the entrainment and determination of activity the latter speculation appears plausible.

In winter many portions of the river are covered with ice, reducing illumination to a very low amplitude. The precision of entrainment may be reduced to a relative co-ordination of activity (Hoffman 1969). This is a very weak, imprecise approximation of entrainment. In the Arctic, apparent free-running periods have been reported in the activity of fish at photoperiod extremes (Müller 1970, 1973). Occasionally there is a shifting or reversal of activity patterns coincident with, and possibly cued by, changes in invertebrate drift patterns. The possibility that small period changes were the result of relative coordination by subtle, weak Zeitgebers has not been investigated. Spectral and light intensity changes in the Arctic have been shown to be effective as either actual or as potential Zeitgebers for some plants and mammals (Teeri 1974, Krull 1976b,c).

Stochastic variations in the aquatic environment can cause rapid and extreme fluctuations in light intensity, reducing its utility as an exact cue. These random fluctuations in light intensity could also cause a reduction in the reliability and effectiveness of proportional intensity effects in entrainment. This could indirectly affect the FRP. The lack of any direct relationships between intensity and tau (see Fig. 34C) does not directly eliminate the possibility of parametric (proportional) actions of photoperiod on the circadian system and the validity of the circadian rule. It may be that only a very limited range of light intensities is appropriate for the circadian relationship to be evident (Kavanau 1962). More significantly, the after-effects of previous photoperiods (LD, LD+t) may override any effects of current environmental conditions, obscuring responses to LL. Steady-state rather than transient FRP values or after-effects are necessary to demonstrate relationships between circadian rhythms and the intensity of constant illumination.

Siegmund and Wolff (1972c) suggested that the FRP of *Leucaspius delineatus* is dependent on the intensity of illumination, as per the circadian rule. However, their use of fish schools, photometric light units, and unorthodox methods of determining period length make it difficult to interpret the results. Schwassman (1971b) reported that FRP of electrical discharge of gymnotid fish, a measure that was highly correlated with activity, showed a strong dependence on light intensity. He suggested that this dependence may be part of a specialized entrainment system utilized in the unique tropical freshwater habitat and behaviours of the electric fish.

The annual variations found in free-running period values of *C. plumbeus* maintained under LD+t entrainment suggests that there is an endogenous circannual variation in the FRP. However, the lack of seasonal changes in tau values of fish entrained under LD photoperiod negates the possibility of there being any direct significant endogenous circannual variations in circadian activity parameters. The results indicate that changes in period lengths are primarily the result of environmentally (twilight) induced alterations in entrainment relationships. In a review of annual rhythms, Menaker (1974) suggested that annual cycles are primarily the result of interactions between daily circadian cycles and annual changes in diel environmental parameters. The seasonal changes in FRP's found in *C. plumbeus* entrained by photoperiods with twilights supports his contention.

Chapter VI

GENERAL DISCUSSION

It has been known for a long time that photoperiods have an important role in timing the behavioural, physiological and ecological patterns of many organisms. However, the roles of the various constituents of photoperiod have not received equivalent attention. In preceding chapters of this [illegible] it was shown that the majority of laboratory and field investigations of rhythms have either ignored or only given cursory attention to twilight periods. Theoretical and descriptive studies analysing the effects of twilights have not considered the complexity of events occurring during dawn and dusk. Seasonal and daily changes in most of the parameters were completely ignored. The results of the present study indicate that there is a need to reconsider the validity of extrapolations from laboratory light-dark cycles to natural photoperiods. The data presented in this thesis indicate that annual changes in twilight must be included in descriptions and analyses of seasonal variations in the entrainment and determination of the free-running period of circadian rhythms.

This study with the diurnally active *C. plumbeus* specifically demonstrated that twilight portions in the natural photoperiods significantly affected the seasonal course of:

1) photoperiodic entrainment (Ψ_{onset}, see Fig. 21);
2) the free-running period, T (see Figs. 33, 34);
3) the seasonal characteristics, length, and distribution of activity sub-components or 'bouts' (Appendix III).

Entrainment of the locomotor activity of lake chub by LD photoperiods that excluded twilights resulted in less precise rhythms. Synchrony to photoperiod was maintained throughout the year, but there were no longer any significant seasonal variations in Ψ and T.

These results clearly demonstrated that the rates of change of various spectral energy components (see Fig. 32) rather than absolute light intensities are the major cues for the entrainment of the beginning of activity. Phasing of the end of activity, Ψ_{offset}, was determined by photoperiod duration and total light intensity present in dusk (see Figs. 25, 26). Comparisons between the overall activity patterns obtained from LD and LD+t entrainment suggest that the presence of twilights may indirectly function to permit the maintenance of the synchronization of activity while progressive, seasonal changes in natural photoperiod are occurring.

This study on the locomotory behaviour of the diurnally active cyprinid, *Couesius plumbeus*, under a wide range of light conditions provided comparative data on circadian rhythms of fish. The data showed that the activity rhythms of fish cannot be directly considered as behavioural or ecological equivalents of the circadian activity parameters of birds and mammals. The latter have been the basis of many generalizations about, and analyses of, circadian rhythms.

Natural history observations have shown that many mammals and birds living in the wild can have significant variations in the times when activity begins and ends. The mechanism cueing circadian rhythms of activity are sufficiently plastic to allow individual animals to modify their behaviours in response to changes in environmental conditions.

The locomotory patterns of *C. plumbeus* obtained from LD+t photoperiods can be considered as 'activity templates' that are cued by the most precise and consistent photic Zeitgebers in the aquatic environment.

In contrast to natural conditions, birds and mammals in captivity show excellent precision in timing. This consistency is at least partially a result of the constant conditions and unique single laboratory behaviour displayed.

Mammalian and avian circadian or entrained rhythmic activity as studied in the laboratory is a single artificial behaviour (Enright 1970). This makes it difficult to carry out any exact ecological and behavioural interpretations. Pohl (1968) has shown that different patterns of rhythmic activity can be obtained for small mammals by varying the type of laboratory outlet available. Swimming and related movements of *C. plumbeus* cannot be considered as equivalent to the wheel running or perch hopping of mammals and birds, respectively. For instance, wheel running activity of hamsters, which is regulated by a highly precise circadian timer (± 2 min) is quite different from generalized body movements which are also circadian but of a much lower precision (Menaker 1976). Therefore in order to obtain more than just a superficial understanding of the temporal patterning and entrainment of the activity of fish, it was considered necessary to have some preliminary knowledge of the full behavioural repertoire.

The dichotomy between the cues for timing the beginning and end of activity led to bimodal and unimodal patterns in Ψ_{onset} and Ψ_{offset}, respectively. These differences in timing suggest that the coupled oscillator model of circadian rhythms elucidated by Pittendrigh (1974) is appropriate for describing the basic locomotory rhythms of *C. plumbeus* and of fish in general. It is conceivable that the beginning and end of maximal activity are associated with separate populations of oscillators that are synchronized with one of the two twilight transitions. These oscillators are mutually synchronized in an integrated circadian system.

Analysis of daily metabolic and morphological processes in plants and insects has shown that 'light-on' (dawn) and 'light-off' (dusk) responses represent separate controls or oscillators within a circadian system (Saunders 1973, Hamner and Hozhisaki 1974).

Studies with vertebrates have also provided evidence for the existence of at least two interacting oscillators in circadian systems. Under extended constant conditions (100-200 days), at supposedly steady-state period values, free-running rhythms of mammals and birds split into two separate uncoupled activity components (Hoffmann 1971; Gwinner 1974).

In a hypothetical system of morning (dawn M) and evening (dusk N) linked oscillators with $T_M > T_{system} > T_N$, the beginning of activity would be synchronized by dawn and the end by dusk (Pittendrigh 1974). The overall pattern is determined by the dominant oscillator, usually that of the more precise M for diurnal animals ($T_M > 24$ hrs) and N for nocturnal animals ($T_N < 24$ hrs). Coupling between the two oscillators would restrict their phase values to a limited range of relationships. Since the oscillators are dawn and dusk coupled, during the course of the year as sunrise and sunset move apart, their phase relationships change. In order to maintain consistent entrainment mutual coupling between the oscillators must counteract external photoperiod changes.

Andreasson (1973) has explained seasonal inversions of activity patterns of arctic fishes on the basis of a coupled oscillator model. He proposed that weak synchronization at photoperiod extremes led to an uncoupling of oscillators. This was followed by a subsequent recoupling in different phase relationships and an alteration of the overall activity pattern.

Pittendrigh and Daan (1976a,b,c) have incorporated these theoretical proposals into an interacting oscillator model of entrainment. They suggested that asymmetric phase responses led to changes in oscillator coupling strength and subsequent alterations in period length. However, this model as well as the other theoretical descriptions of entrainment are based solely on the results obtained from LD photoperiods. All models and theoretical descriptions proposed to date have the assumption that similar cues are used for timing the beginning and end of entrained circadian rhythms. The present results with twilight vitiated this assumption. The results obtained with *C. plumbeus* do indicate, however, that the entrained state, which is twilight influenced or dependent, is a major determinant of the free-running period.

Examination of activity under photoperiods with and without dawn (see Fig. 31) indicated that Ψ_{onset} and the beginning of activity determined the basic characteristics of the overall entrained form. Upon first examination there appears to be a contradiction present: Ψ_{onset}, cued by twilight, has a bimodal annual pattern, while T and bout lengths have a unimodal pattern consistent with seasonal changes in photoperiod duration. This implies that period and phase values, for onset at least, are not related in a direct linear manner. However, the net relationship after oscillator interactions of the various behavioural components could very well be linear. In an analysis of the time course of entrainment, Kramm (1973, 1975) indicated that there was a non-linear relationship between T, the entrained period and Ψ. He suggested that complete entrainment is a unique phenomenon that is rarely attained. Rather, a limit cycle around a steady-state value is obtained. The net result could involve a utilization of proportional and differential cues, whose interactions, directly or indirectly, lead to linear variations in Tau.

A circannual modification of the oscillator model has been proposed (Gwinner 1975). One oscillator was supposed to be related to the 24 hr Zeitgeber, primarily non-parametric in action, and the other under the control of seasonal factors. These were postulated to result in an endogenous annual pattern.

Ashan (1966a,b) has shown what he termed 'endogenous' annual changes in testicular and to a lesser degree ovarian activity of *C. plumbeus*. Menaker (1974), as discussed earlier, points out liabilities of assuming that so-called endogenous annual rhythms are functional equivalents of circadian cycles. He suggests that annual changes in daily rhythms can be broken down into environmentally induced variations. Exogenous stimuli, either physical or biological in origin, may systematically alter the repetitive patterns of photoperiodically induced entrainment.

Ashan (1966a) also showed that testicular development was primarily cued by temperature, with photoperiod acting only as an ancillary factor. In the present experiments identical annual temperature cycles were employed with LD and LD+t. In all likelihood the temperature cycles induced similar levels of gonadal development and gonadotrophin activity. This suggests that annual patterns of gonadal development and hormonal activity were not, by themselves, the immediate cause of seasonal variations in the activity parameters of chub. However, there is an increasing amount of evidence indicating that hormones have a regulatory action in vertebrate circadian systems. Testosterone and estradiol have been shown to shorten the period length of, and induce phase shifts in, circadian wheel running activity of hamsters (Pittendrigh and Daan 1976a; Morin et al. 1977b). It is possible that for *C. plumbeus* the attainment of a precise Ψ relationship under twilight cues leads to a detectable expression of hormonally induced oscillator interactions. It is conceivable that if a single behavioural parameter were examined, its tau values might show some annual fluctuations under LD photoperiods. However, since the locomotory activity of fishes is made up of a number of behaviours it is highly likely that complete synchrony is necessary before any seasonal variations in mean tau values become evident.

Under twilight entrainment short-term activity components or 'bouts' displayed seasonal variations in their distribution and parameters (Appendix III). Annual variations in bout length may be considered as either the direct or indirect results of seasonal changes in hormonal levels. Results from studies of behavioural sequences and composition of the activity of cichlids suggest that changes in hormonal levels could lead to long-term alterations in stochastic, behavioural characteristics (Heiligenberg 1973; 1974; Metz 1974). These modifications would be demonstrated as changes in the behavioural make-up of each 'bout' and the overall 'bout' durations and distributions (see Appendix III). However, complete expression of sub-component distribution probably requires concurrent changes in the circadian oscillator system and indirectly photoperiodic entrainment.

Thus three general conclusions emerge from the results of this investigation:

1) twilights of both dawn and dusk are necessary constituents of the photoperiod for the full expression of seasonal variations in activity rhythms and their various parameters, specifically entrainment phase relations and the free-running period;

2) specific components of twilights (dawn primarily in this case), such as rates of change of spectral energy, rather than absolute light levels are utilized as cues for the timing or phasing of activity, and

3) it is necessary to reappraise any proposed mechanism for the control of seasonal changes in photoperiodic entrainment and tau values in which seasonal changes in twilight or its constituents have been overlooked.

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APPENDIX I

CHARACTERISTICS OF COLLECTING AREAS

The North Saskatchewan is a large northern river 50-60 m wide with a mean depth of 1M, located in a glacial drift valley. It is characterized by steep banks at the outside of turns and gravel plains inside. The latter were present in the collecting areas. Whitemud is a relatively turbid creek that flows into the North Saskatchewan depositing mud and silt. Specific limnological features of the river and environs are discussed by Paterson (1966). Physical and chemical parameters, vegetation and invertebrate composition present during the collecting times (1974, 1975, 1976) are available (unpublished records, University of Alberta). Sample records of seasonal variations in water temperature, turbidity, and oxygen levels are shown in Figure 1. Total numbers and relative species composition of fishes obtained at the collecting periods are listed in Table 1.

Fig. 1. Mean monthly water temperature (°C), turbidity (Jackson Turbidity Units), and dissolved oxygen levels (ppm) at the collecting sites in the North Saskatchewan River during 1976. All values were obtained from a depth of 10-15 cm.

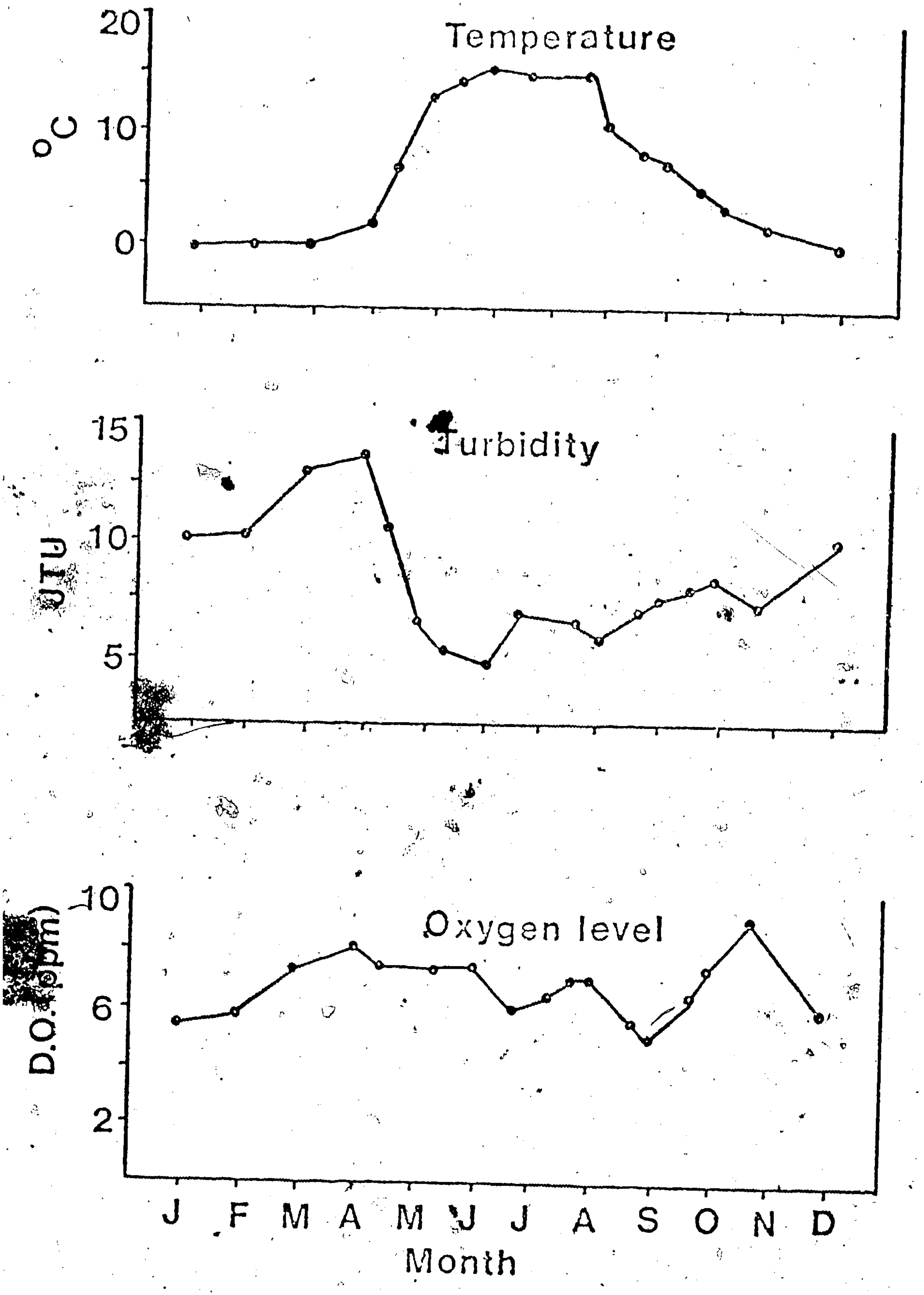

Temperature
°C
20
10
0
Turbidity
JTU
15
10
5
Oxygen level
D.O. (ppm)
10
6
2
J
F
M
A
M
J
J
A
S
O
N
D
Month

Table 1. Composition and relative abundance of fishes at collecting sites in the North Saskatchewan River. Values are for 1974-1976.

Species of fish	Percentage (%) occurrence of various species of fish[1]																							
						June[2]				July[2,3]				Aug.[2,3]				Sept.						
	Jan.	Feb.	Mar.	Apr.	May	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	Oct.	Nov.	Dec.
Hybopsis gracilis	01	-	05	04	05	-	-	01	01	01	03	01	-	02	02	01	01	-	-	-	-	-	-	-
Couesius plumbeus	43	10	06	07	06	02	02	02	01	05	05	10	01	05	01	04	05	03	05	06	02	04	08	13
Notropis atherinoides	13	04	01	05	01	01	02	02	05	08	05	-	03	-	01	03	02	01	01	05	03	01	03	05
Notropis blennius	08	03	05	05	05	03	02	-	04	05	02	-	04	01	03	05	05	02	03	05	05	02	01	03
Notropis hudsonius	03	02	02	03	05	03	01	-	02	01	01	01	01	02	04	05	05	02	01	04	04	02	02	04
Rhinichthys cataractae	01	05	06	10	10	09	10	30	15	20	07	01	02	07	06	05	06	02	02	05	01	03	05	02
Pimephales promelas	-	-	01	01	01	02	02	01	02	-	01	09	03	-	01	-	01	-	-	-	-	01	-	01
Catostomus catostomus	17	41	28	24	27	30	12	22	25	20	40	40	40	39	35	35	39	48	41	40	42	47	40	40
Catostomus commersoni	13	35	41	31	30	26	15	20	25	20	35	35	40	43	45	39	34	42	46	35	43	40	38	30
Culaea inconstans	-	-	01	05	05	16	48	02	01	-	-	02	01	-	01	02	01	-	-	-	-	-	01	-
Percopsis omiscomaycus	01	-	04	05	05	08	06	20	19	20	01	01	05	01	01	01	01	-	-	-	-	-	02	02
Total no. of fish	250	150	225	240	350	800	600	400	300	500	1100	900	800	2200	1400	2000	2500	1200	800	600	500	350	250	125

[1] Only the most abundant species are listed. Single representatives of species such as *Esox lucius*, *Lota lota* and *Stizostedium vitreum* are omitted.

[2] Weekly values are listed. These indicate changes in species composition caused by spawning movements.

[3] Young of the year comprise 50-75% of the total fish.

APPENDIX II

Table 1. *Coucesius plumbeus* used in experimental procedures.

CODE NUMBER	SEX	AGE yrs	CAPTURE DATE	LABORATORY PROCEDURES/EXPERIMENTAL MANIPULATIONS		
				PHOTOPERIOD		
A1	M	1+	1974	LD, DD		
A2	F	2+	1974,	LD, DD		
A3	M	3+	•	LD, DD		
A4		1+		LD, DD		Preliminary procedures: demonstration of diel activity, FRP, and entrainment by LD
*A5	M	2+	•	LD, LL		
A6	M	1+	•	LD, LL		
A7	F	3+	•	LD, LL		
A8	M	2+	•	LD, DD		
*A9	F	1+	•	LD, DD		
A10	M	4+	1974	LD, DD		
B1-B5	M	1+	1974			
B5-B10	F	2+				
B11-B15	M	3+				
B16-B20	F	1+		Natural outdoor		Behaviour and activity sub-component determinations Fish captured and immediately observed
B21-B25	M	2+				
B26-B30	F	3+				
B31-B36	M	2+	1974			
B40-B45	F	2+	1974, 1975			
B46-B59	M	2+				
B51-B55	F	2+				
B56-B60	M	1+				
B61-B65	F	2+		Natural outdoor		Behaviour and activity sub-component determinations Fish captured and maintained outdoors
B66-B70	M	3+				
B71-B75	F	1+				
B76-B90	M	2+	1976			
*B81-B90	F	3+	1976			
B101	M	2+	1974	LD	Phase	
B102	F	3+	•	LD+t, DD	Phase, FRP	
B103	M	1+	•	LD	Phase	
B104	F	2+	•	LD, DD	Phase, FRP	
B105	M	3+	•	LD+t	Phase	
B106	F	3+	•	LD	Phase	
B107	F	2+	•	LD	Phase	
B108		1+	•	LD+t	Phase	
*B109	M	1+	•	LD	-	Phase relationships, FRP, activity sub-components, determined over 1974 seasonal photoperiod cycle
B110		1+	•	LD+t	Phase	
B111	M	2+	•	LD+t, DD	Phase, FRP	
B112	F	3+	•	LD	Phase	
B113		1+		LD+t, DD	Phase, FRP	
B114	M	3+	•	LD	Phase	
B115	F	1+	•	LD+t, DD	Phase, FRP	
B116	F	2+	•	LD+t	Phase	
B117	F	3+	•	LD+t, DD	Phase, FRP	
B118	M	2+	•	LD+t	Phase	
*B119	M	2+		LD	-	
B120		1+	•	LD+t	Phase	
B121	F	2+	•	LD+t, DD	Phase, FRP	
B104	M	1+	1974	LD+t		
B105		-		•		
B107	M	2+	•	•		
B108	F	1+	•	•		
B109	M	2+	•	•		
B110	F	3+	•	•		
B111	M	2+	•	•		
B112		1+	•	•		Fish captured, placed under seasonally appropriate LD+t photoperiods and their phase relationships were determined
*B113		1+	•	•		
B115	M	2+	•	•		
B127	F	3+	•	•		
B131	F	2+	•	•		
B132	M	1+	•	•		
B133	M	2+	•	•		
B134	M	3+	1974	LD+t		

* Fish died during course of experiment and results were not used

(Cont'd)

Table 1 (Cont'd).

CODE NUMBER	SEX	AGE yrs	CAPTURE DATE	LABORATORY PROCEDURES/EXPERIMENTAL MANIPULATIONS	
				PHOTOPERIOD	
B135	F	3+	1974	DD	Fish captured and immediately placed under DD for determination of their FRP values. Annual cycle of natural photoperiod examined
B136	M	2+	•	•	
B133	F	2+	•	•	
B139		1+	•	•	
B141		1+	•	•	
B142		1+	•	•	
B147	M	2+	•	•	
B152	F	3+	•	•	
B153	M	3+	•	•	
B154	F	2+	•	•	
B155	M	2+	•	•	
B156	F	2+	1974	DD	
C6	M	3+	1975	LD+t, DD	Fish kept under seasonally appropriate LD+t photoperiods Phase relationships, behaviour and FRP under DD were determined
C7	F	2+	•	•	
C12	M	2+	•	•	
C15	F	3+	•	•	
C18		2+	•	•	
C22	F	3+	•	•	
C27	M	2+	•	•	
C29	M	2+	•	•	
C32	F	2+	•	•	
C38	M	2+	•	•	
C40	F	2+	•	•	
C41	M		1975	LD+t, DD	
C5	M	2+	1975	LD, DD	Fish kept under seasonally appropriate LD photoperiods. Phase relationships, behaviour, and FRP under DD were determined
C8	F	3+	•	•	
C9	M	1+	•	•	
C16	M	1+	•	•	
C23		1+	•	•	
C25	F	2+	•	•	
C28	M	3+	•	•	
C30	M	3+			
C33	F	2+			
C34	M	1+	•	•	
C35		1+	•	•	
C36	M	2+	1975	LD, DD	
C1	M	2+	1975	LD+t	Fish captured and immediately placed under LD+t for phase relationship determinations
C2	F	1+	•	•	
C3	M	2+	•	•	
C4		1+	•	•	
C10	F	2+	•	•	
C11	M	3+	•	•	
C17	F	3+			
C19	M	2+	•	•	
C20	F		•	•	
C21	M	1+	1975	LD+t	
C26	F	2+	1975	DD	Fish captured and immediately placed under DD for FRP determinations.
C31	M	2+	1975	DD	
C37	F	3+		DD	
C39	M	2+		DD	
C40	M	1+		DD	
C42	F	2+	1975	LD	Spectral thresholds and sensitivities determined
C46	M	3+		LD	
C48	F	2+		LD	
C49	M	2+		LD	
C51	F	3+		LD	

(Cont'd)

Table 1 (Cont'd).

CODE NUMBER	SEX	AGE	CAPTURE DATE	LABORATORY PROCEDURES/EXPERIMENTAL MANIPULATIONS	
	yrs			PHOTOPERIOD	
D2	F	2+	1976	LD+t.DD	Fish were captured and placed under seasonally appropriate LD+t photoperiods. Phase relationships behaviour and FRP under DD were determined
D5	M	2+	.	.	
D6	F	2+	.	.	
D8	M	3+	.	.	
D9		1+	.	.	
D11	F	4+	.	.	
D13	M	3+	.	.	
D14	F	2+	.	.	
D15	M	3+	.	.	
D17		1+	.	.	
D18	F	2+	.	.	
D20	F		1976	LD+t,DD	
D3	M	2+	1976	LD,DD	Fish were captured and placed under seasonally appropriate LD photoperiods.Phase relationships behaviour and FRP under DD were determined
D4	F	3+	.	.	
D7		1+	.	.	
D10	M	2+	.	.	
D12	F	3+	.	.	
D16	M	4+	.	.	
D19	F	2+	.	.	
D22		1+	.	.	
D23	M	3+	.	.	
D24	F	2+	.	.	
D25	M	2+	.	.	
D28	M	2+	1976	LD,DD	
D29	M	3+	1976	DD	Fish were captured and immediately placed under DD for FRP determinations,and activity sub-components
D30	F	2+	.	DD	
D31	M	3+	.	DD	
D34	M	2+		DD	
E2	M	2+	1976	LD+t	
E3	F	1+	.	LD+t	
E4		2+	.	LD+t	
E5	F	3+	.	LD+t	
E6	M	3+	.	LD+t	
E9	F	2+	1976	LD+t	Dusk was removed from LD+t
E12	M	3+	.	LD+t	
E13	F	4+	.	LD+t	
E14	M	1+	.	LD+t	
E16	F	2+	.	LD+t	
E10	F	2+	1976	LD,DD	Dawn was removed from LD+t
E11	M	3+	.	LD,DD	
E15	F	4+	.	LD,DD	
E17	M	1+	.	LD,DD	
E19	F	2+	.	LD,DD	
E20	M	3+	.	LD,DD	
E23	F	2+	1976	LD,DD	
E25	F	3+	1976	LL	Affect of LL on the FRP
E27	M	2+	.	LL	
E28	F	3+	.	LL	
E30	F	1+	.	LL	
E31	F	1+	.	LL	

APPENDIX III

ANALYSIS OF THE SUB-COMPONENT OF LOCOMOTORY ACTIVITY

If swimming behaviour or activity can be described as a simple stochastic (random-decision) process, then locomotion and its frequency-duration distribution should occur as a random ensemble. A mathematical description for a completely random series of events is a Poisson distribution, represented by an exponential curve.

In undertaking any stochastic analysis of behaviour sequences, a preliminary step is to test for the goodness of fit of the interval distributions to an exponential distribution (Van der Kloot and Morse 1974). A number of statistical methods can be used to test for the goodness of fit of data sets to empirical distribution functions. The main one used in this study was Durbin's method (Lewis 1965).

Durbin's method: This test is powerful in distinguishing between an exponential (random) and any number of vaguely specified non-random alternative distribution functions. It is the test that is least affected by non-stationarity in the original time-series. Non-stationarity is indicated by the trends present in the original time series and autocorrelation transforms before linearization. Durbin's method employs a transformation of the original time series. Time intervals (activity durations) from the frequency histograms are arranged in ascending order: $x_1, x_2 \ldots\ldots\ldots x_n$ and then transformed by

$$U_i = \frac{1}{T_o} [x_1 + x_2 + \ldots\ldots\ldots (n+2 - 1)]$$

where: $i = 1,2,\ldots\ldots\ldots n$

T_o = total time of the set

U_o = transformed values used for analysis.

The transformed set U_i is graphed against 1/n giving a survivorship plot. If the original distribution is exponential, a straight line with a slope of 1 is generated. The goodness of the data set fit to the predicted line for an exponential process (Poisson) is estimated by the Kolmogoroff-Smirnov test using the D_N statistic (Sokal and Rohlf 1969). The results of the tests are listed in Table 1.

An Ln-survivor curve was obtained by arranging behavioural (activity duration) events in an ascending series: $x_1 < x_2 \ldots\ldots < x_n$ as a function of i,

where $x_i \ldots\ldots x_n$ are interval sizes with n being maximum
$1 = 1, \ldots\ldots n$
N = number of frequency bands in a certain class.

For a Poisson prediction, the Ln-survivor curve is a straight line of slope 1, with an intercept 0. The degree of data deviation from the line provides an indication of deviation from a random form and allows comparisons between different data sets.

The results from Durbin's test (Table 1) and Ln-survivor curves (Fig. 1) reveal that the distribution histograms for activity entrained by LD+t, LD and to a certain extent from DD (rhythmic portions) differs significantly from a Poisson distribution.

The general type of theoretical distribution most similar in appearance to these histograms is a Gamma (γ) distribution. This is a continuous 2-parameter function of the following form (Lewis 1965):

$$F(x) = \frac{1}{\beta^{\alpha+1}\overline{|\alpha+1}} \qquad x^{\alpha} e^{-x/\beta}$$

where F(x) = γ distribution
α = skewing factor with $\alpha > -1$; if $\alpha = 0$, an exponential function is formed

β = scaling factor with $\beta>0{\cdot}0$

$\alpha\beta$ = mode

Γn = Gamma (γ) function

$= \int_0^\infty x^{n-1} e^{-x}\, dx$

n = interval class

x = component of the series.

The Gamma function can be considered as an exponential function rendered non-random by a deterministic component.

The observed distributions (Fig. 1) were not significantly different from the theoretical Gamma forms at the 5 per cent level (Kolmogoroff-Smirnov test). Maximum likelihood estimates for α and β in the Gamma distributions are provided by Johnson and Kotz (1970). Expected values were found by using Pearson's (1922) tabulation of incomplete Gamma functions. The exact fitting distributions are described by Lewis (1965). Distribution values for different photoperiodic histories are listed in Figure 1.

Results activity under photoperiods with twilights (LD+t)

Under simulated natural photoperiods and twilight lighting combinations (LD+t) there are seasonal (annual) changes in the total amount of, duration, and distribution of chub locomotory activity bouts. Representative frequency histograms and Ln-survivor curves are shown in Fig. 1. Alterations are shown as differences in the shape of the Ln-survivor curve and in the value of the β term in the frequency distribution. Beta (β) is a function of the duration of the duration of the mean activity burst, while alpha (α) is a scaling factor that varies with group size and amplitude of motion. Seasonal changes in water temperature affect α rather than β.

During winter (Nov-Mar) the length of the average activity bout is shorter than, and the total percentage of activity is reduced as compared to, the summer activity components (Fig. 1). At photoperiod extremes (summer, winter solstices) the total amount and durations of activity approach an inflection point.

Activity under light-dark (LD) cycles

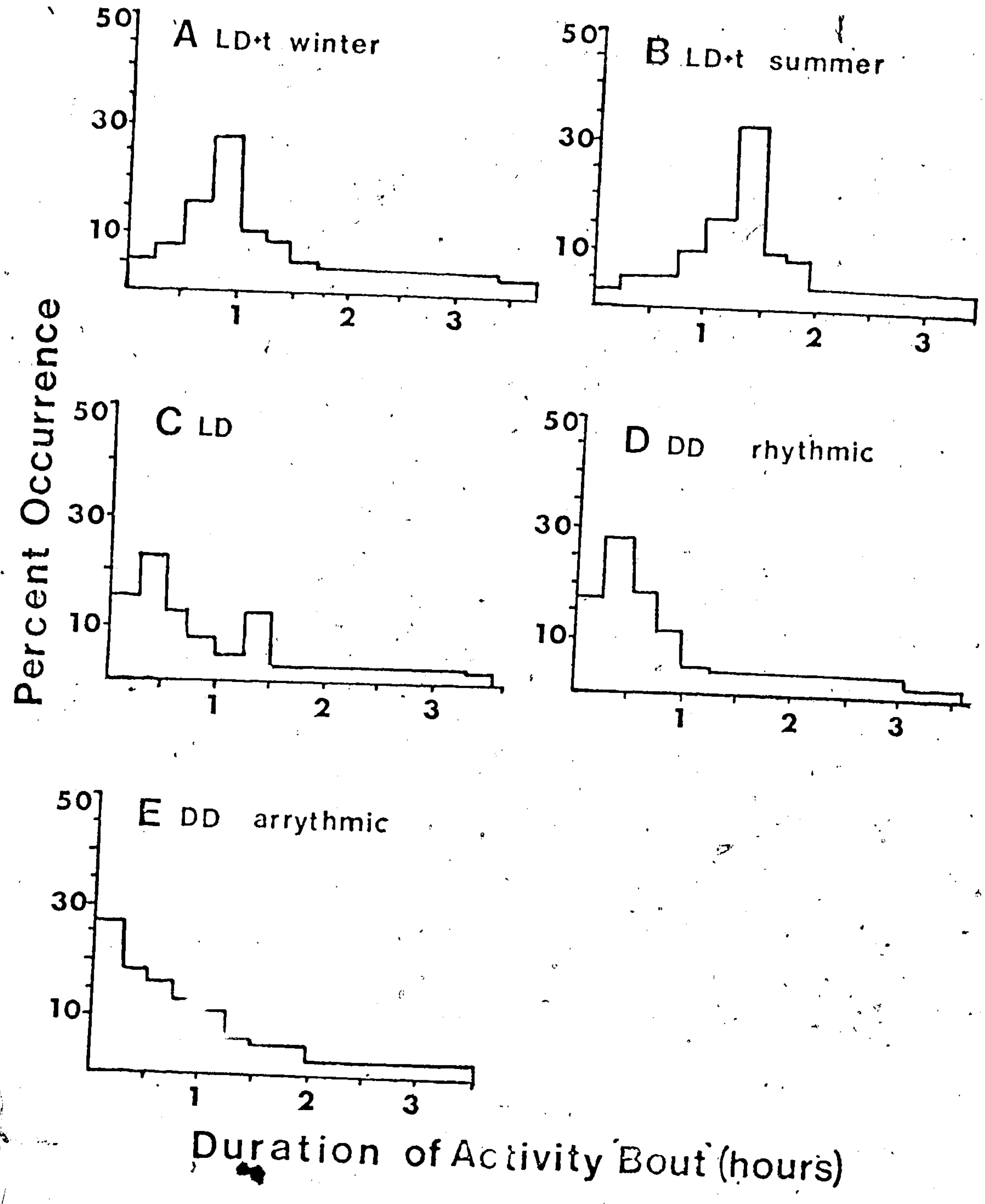
The frequency distributions of activity bouts obtained under on-off LD cycles (excluding twilights) are shown in Figure 1. These sub-components distributions more closely approximate exponential forms than those recorded under LD+t. The Ln-survivor curve is closer to linearity, but is still significantly different from a random state (Table 1). A distribution with a lower β value and a broad mode αβ is obtained.

Mean monthly bout lengths for lake chub held under LD and LD+t are shown in Figure 2 (sample sizes are indicated in the legend). Under LD+t, twilight entrainment, there was a significant annual pattern or cycle in mean bout length. No significant differences were evident from LD conditions.

Fig. 1. Frequency-distribution histograms and Ln-survivor curves of *Couesius plumbeus* locomotory activity bouts. The experimental conditions for each set of histograms are as follows:

	Photoperiod	Time of year	Distribution of characteristics
A	LD+t	summer (May-Sept)	gamma; $\alpha=.59$, $\beta=3$
B	LD+t	winter (Oct-Feb)	gamma; $\alpha=.38$, $\beta=2.1$
C	LD	winter (Oct-Feb)	gamma; $\alpha=.58$, $\beta=1.2$
D	DD	circadian portion	gamma; $\alpha=.24$, $\beta=.42$
E	DD	arrythmic portion	exponential

Each histogram and Ln-survivor curve is based on the activity of 10 lake chub over 3 days.


A LD+t winter
B LD+t summer
C LD
D DD rhythmic
E DD arrythmic
50
30
10
1
2
3
Percent Occurrence
Duration of Activity Bout (hours)

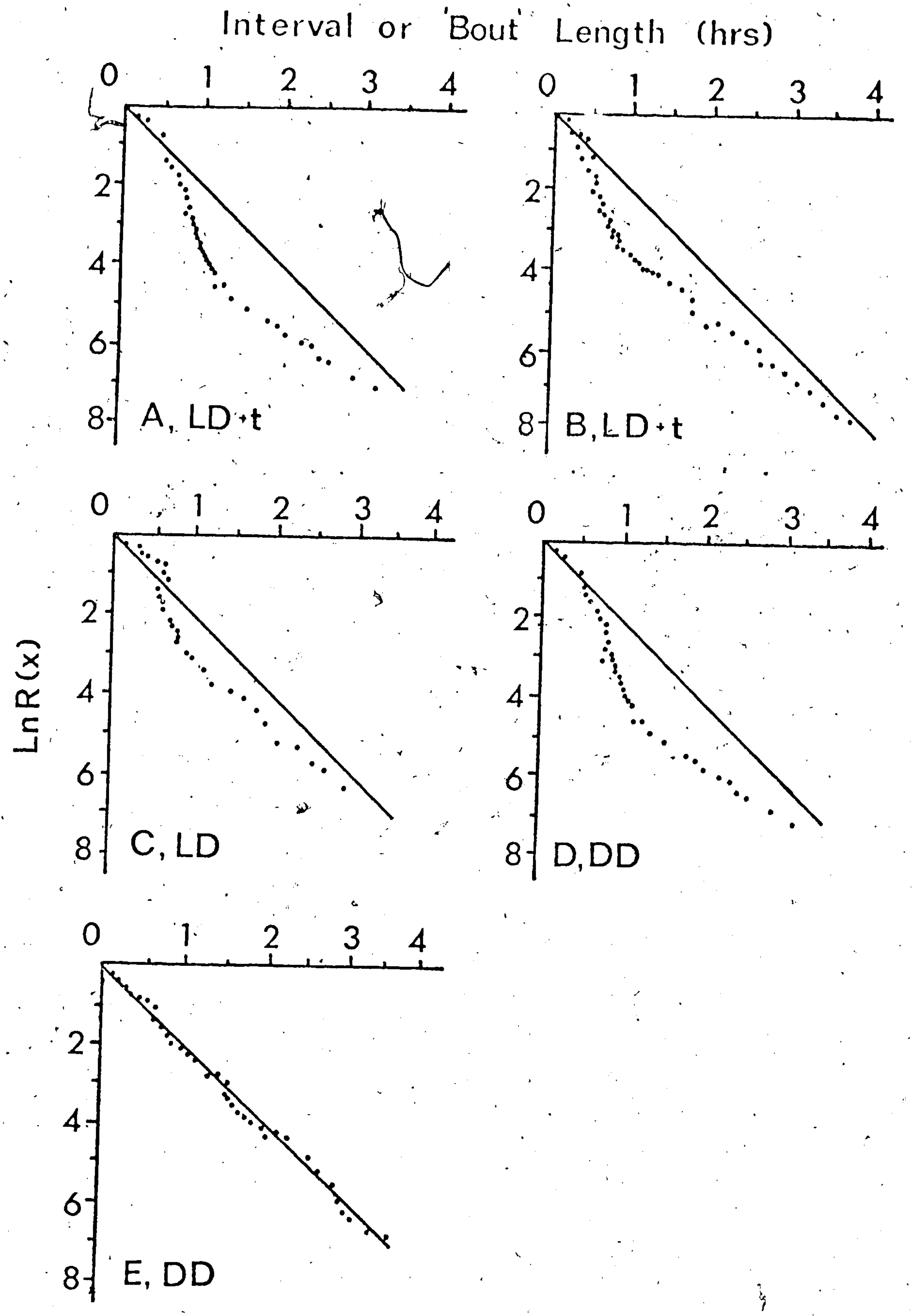
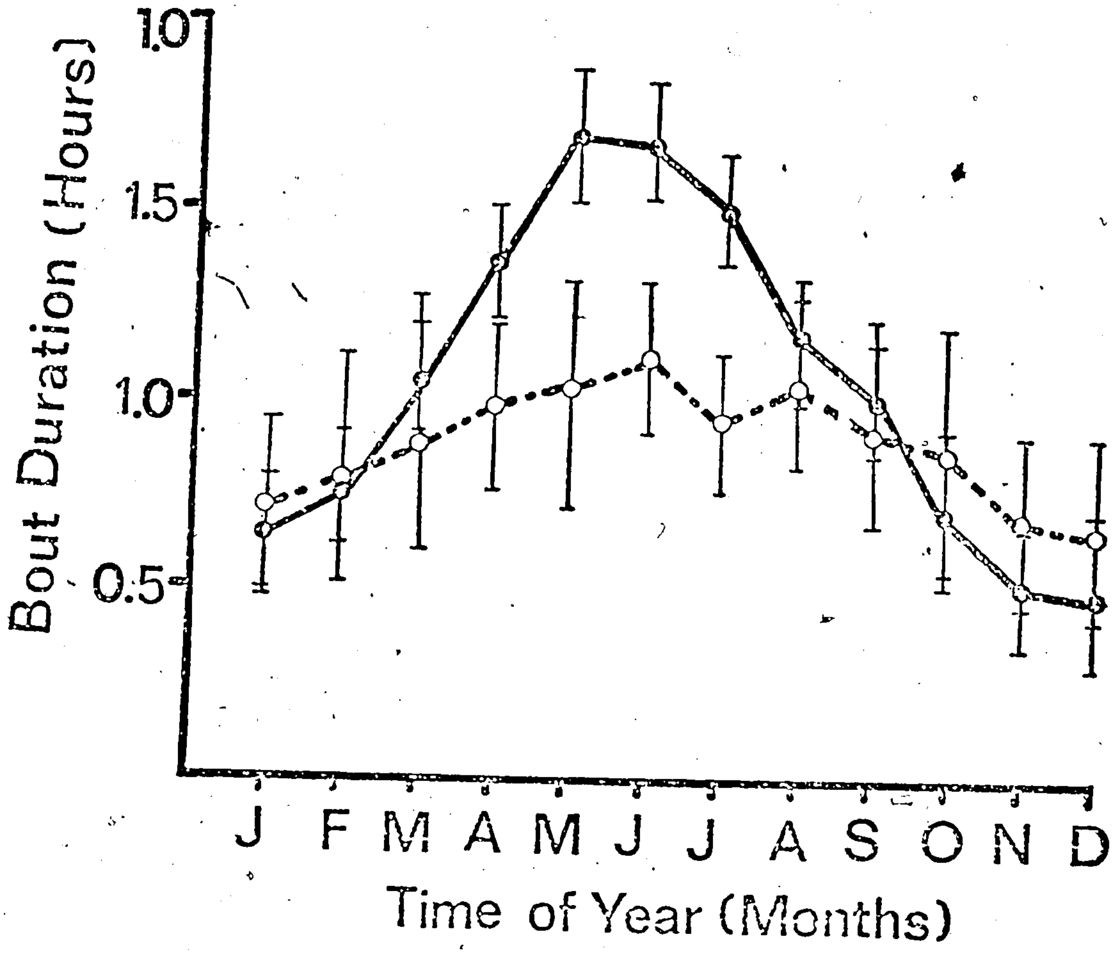

Interval or 'Bout' Length (hrs)
0
1
2
3
4
LnR(x)
2
4
6
8
A, LD+t
B, LD+t
C, LD
D, DD
E, DD

Table 1. Kolmogoroff-Smirnov test for goodness of fit of chub activity bout frequency distributions to an exponential (random) function. Activity bout distributions were transformed according to Durbin's method, as described in the text. Interval distributions having a significant $D_{\sqrt{N}}$ value follow a non-random function. All probability values are valid for individual fish and pooled sample intervals. Number of fish in each sample are in parentheses.

Experimental Photoperiod	Intervals	$D_{\sqrt{N}}$	P
Light-dark cycle with twilight (LD+t)	15 (34)	0.89	<.001
Light-dark cycle (LD)	15 (32)	0.63	<.05
Constant conditions (DD) (circadian component)*	15 (12)	0.54	<.05
Constant conditions (DD) (arrythmic component)*	15 (12)	0.45	>.05 (N.S.)

*Activity bout distributions were determined before and after (arrythmic) extinction of the circadian rhythm.

Fig. 2. Seasonal variations in the mean activity bout lengths of *Couesius plumbeus*. Vertical lines represent one standard error. ——— represents LD+t entrainment (n=21); ----- represents LD entrainment (n=21).



Bout Duration (Hours)
1.0
1.5
1.0
0.5
J
F
M
A
M
J
J
A
S
O
N
D
Time of Year (Months)

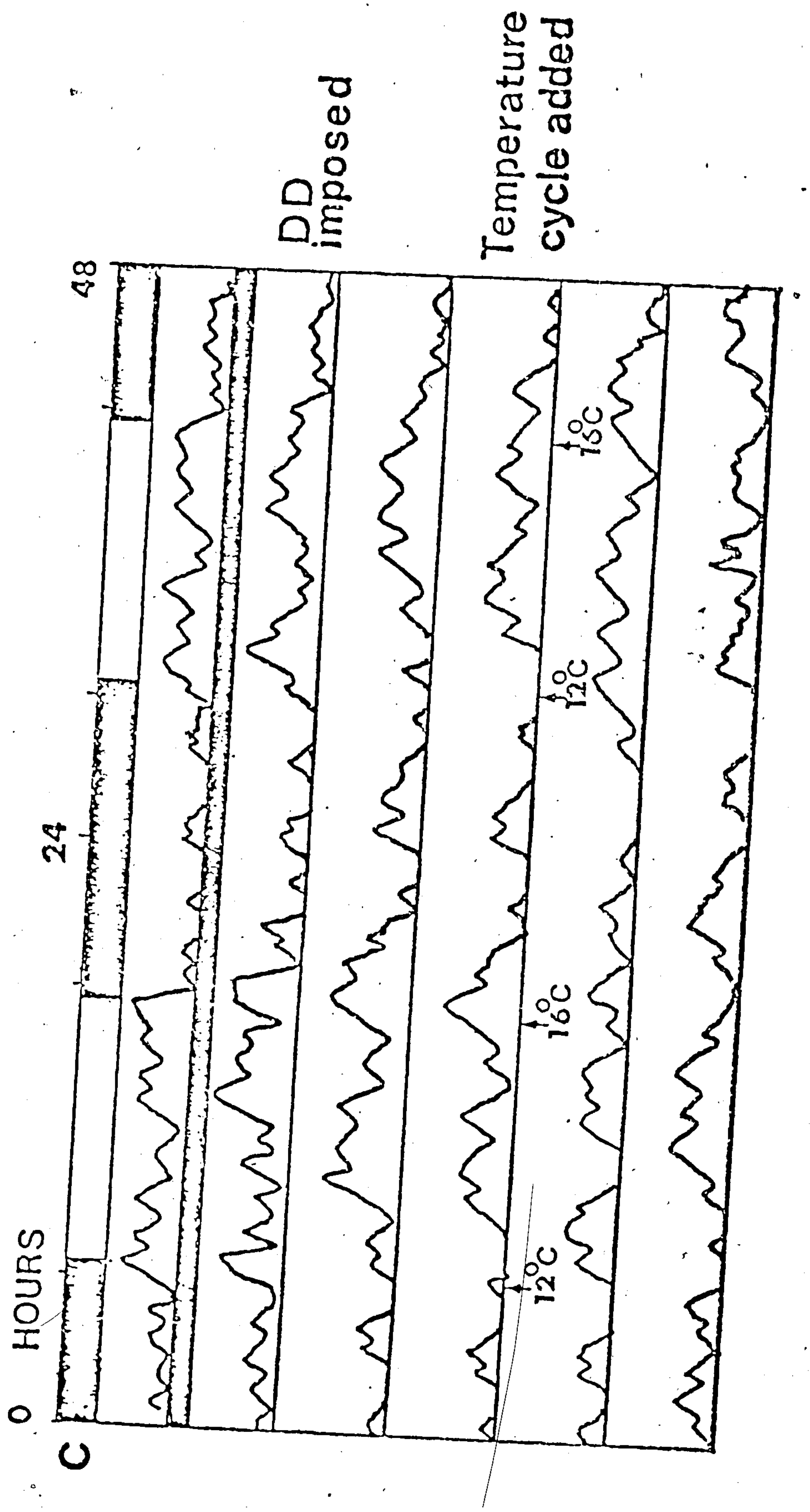
APPENDIX IV

INEFFECTIVENESS OF TEMPERATURE CYCLES AS ZEITGEBERS

Temperature cycles have been shown to act as entraining stimuli for poikilotherms (Schwassman 1971a). Therefore it was necessary to establish that the activity of fishes was not entrained by temperature cycles associated with either water inflow or heating effects of the lights. The general methods and materials section describes two methods used to reduce temperature fluctuations; air cooling of lights and a regulator to control water inflow temperatures. These procedures effectively eliminated any regular temperature cycles. Occasional temperature fluctuations (±2°C) caused by aquaria malfunctions were unavoidable. These changes altered the amplitude of activity rather than the period.

Conclusive evidence that temperature cycles were not entraining fish activity was obtained in the following experiments (see Fig. 16C). Chub were kept under DD and constant water temperature until an apparently arrythmic state was reached. Then a 12:12 (hrs) temperature cycle (12°C:16°C) was introduced for several periods. No indication of periodicity was apparent in the spectra or periodograms analyses. Replicates (4) of temperature cycles were examined at different times of the year. There was no indication of induced periodicity in any of the activity records. A 4°C range was used because that was the maximum mean daily fluctuation that fish were exposed to in the tank. An example of the results obtained with a single fish are shown in Figure 1. The procedures were repeated with 10 different lake chub throughout the year. Similar results to the one indicated were obtained at all times.

Fig. 1. Locomotory activity of a single *Couesius plumbeus* under a 12:12 LD photoperiod followed by constant darkness and then by 12:12 (hr) 12°C:16°C temperature cycle.


C
0
HOURS
24
48
DD imposed
Temperature cycle added
12°C
16°C
12°C
16°C

APPENDIX V

COMPONENTS OF THE DIMMING CIRCUIT

$C_1=C_3=$ 0.1 µfd, 250V

$C_2=C_4=$ 0.1 µfd, 50V

$D_1=$ IN758

$L_1=$ 150 watt

$R_1=$ 6800Ω, 1 watt

$R_2=$ 510Ω, 2 watt

$R_3=$ 5K

$R_4=R_6=R_9=$ 150 KΩ, 1 watt

$R_5=R_7=R_8=$ 120Ω

$R_{10}=$ 470Ω

$R_{11}=R_{16}=$ 22KΩ, 0.5 watt

$R_{12}=R_{13}=R_{14}=$ 170Ω, 0.5 watt

$R_{15}=R_{17}=$ 670Ω, 1 watt

$R_{18}=R_{19}=$ 180Ω, 1 watt

$S_1=$ 120 V

$S_2=S_3=S_4=$ 60 V

$Th_1=Th_2=$ 6RS56P85

$TR_1=$ 2N1047

$TR_2=$ 2N548

$Z_1=Z_2=$ 1M2075